Occupational Asthma in Greenhouse Flower and Ornamental Plant Growers

Eduard Monsó, Ramón Magarolas, Isabel Badorrey, Katja Radon, Dennis Nowak, and Josep Morera

Department of Pneumology, Hospital Germans Trias i Pujol, Badalona; Pneumology Unit, Hospital Mare de Deu de la Salut, Tarragona, Catalonia, Spain; and Institute and Outpatient Clinic for Occupational and Environmental Medicine, Faculty of Medicine, Ludwig Maximilians University, Munich, Germany

The objective of this study was to determine the prevalence of occupational asthma and sensitization to workplace allergens in greenhouse flower and/or ornamental plant growers. A random sample of 40 growers who cultivated such crops and had participated in the European Farmers’ Study was selected for a cross-sectional study that included (1) greenhouse characteristics and air contaminants (dust, endotoxin, and microorganisms), (2) respiratory symptoms, (3) occupational asthma diagnosed by bronchial provocation challenge, and (4) sensitization to workplace flowers or molds. Associations among respiratory symptoms, sensitization, greenhouse characteristics, and air contaminants were analyzed by logistic regression modeling. Thirty-nine growers agreed to participate (response rate, 97.5%). Bronchial provocation challenge confirmed occupational asthma in three workers (7.7%), all of them sensitized to workplace flowers or molds. No cases of occupational asthma were found among nonsensitized growers. Poor ventilation proved to be a marginal risk factor for wheezing (air velocity: odds ratio, 0.11; 95% confidence interval, 0.01–1.04).

Sensitization to flowers or molds was found in 13 of 38 growers (34.2%) but was not related to greenhouse characteristics. We conclude that one-fourth of greenhouse flower and/or ornamental plant growers sensitized to workplace flowers or molds have occupational asthma, a disease that is suffered by 8% of the growers who cultivate these crops.

Keywords: flower; greenhouse; molds; occupational asthma; sensitization

CROP farming is a risk factor for respiratory symptoms, and more specifically, for bronchial asthma (1–3). Agriculture workers are currently exposed to a variety of respiratory irritants and sensitizers, such as pollens, molds, endotoxin, mites, and pesticides (4–6), and the exposure to field crops for long periods has been considered a risk factor for respiratory symptoms (7). The cultivation of greenhouse flowers and/or ornamental plants emerged as a significant risk factor for bronchial asthma in the European Farmers’ Study (8), which focused on the prevalence and risk factors for airway obstruction in farmers and agriculture workers (8–10). Previous work on greenhouse growers has found a high prevalence of respiratory symptoms when compared with control populations (11), and occupational asthma to *Tetranychus urticae*, a macroscopic mite that may infest a wide range of plants, has been occasionally reported in these growers (12–14). Such reports, however, have focused on greenhouse workers cultivating vegetables and tomatoes and have included only flower growers cultivating *Dianthus caryophyllus*. Greenhouse flower and/or ornamental plant growers, however, cultivate a wide range of flowers and are also exposed to molds that are well known to be respiratory sensitizers, and these exposures may be related to the appearance of occupational asthma.

The objective of the present study was to measure greenhouse air contaminants and to assess the prevalence of occupational asthma and sensitization to workplace allergens among the greenhouse flower and/or ornamental plant growers who participated in the European Farmers’ Study. In addition, we also sought to identify greenhouse-related risk factors for wheezing and sensitization to workplace allergens in this population.

METHODS

Design and Population

As part of the European Farmers’ Study (European Union Concerted Action “Prevalence and Risk Factors for Airway Diseases in Farmers”) (9), we performed a cross-sectional study of greenhouse characteristics, air contaminants, prevalence of occupational asthma, and sensitization in flower and/or ornamental plant growers working inside greenhouses. Enrollment details of the random sample of crop farmers from four European countries (Denmark, Germany, Switzerland, and Spain) who participated in the European Farmers’ Study have been reported elsewhere (response rate, 85.3%) (8). Seventy-three of the 85 flower/orchamental plant growers working inside greenhouses worked in Spain (85.9%), and for the present study a random sample of 40 growers was selected from this population.

Questionnaire

A questionnaire with 10 items was used. Five questions concerned age, sex, smoking habits, wheezing, and asthma attacks within the past year, obtained from the European Community Respiratory Health Survey questionnaire (15, 16). Five questions about occupational details asked the subject to specify the cultivated flowers or ornamental plants, the number of hours spent working inside greenhouses or storage areas every day, and the method and frequency of pesticide use.

Field Measurements

Area (m²), temperature (°C), relative humidity (%), air velocity (m/s), and CO₂ concentration (ppm) were measured in the greenhouse when the worker began to work in the morning. Exposure to total dust, endotoxin in total dust, and microorganisms was determined at the breathing zone by means of a personal sampler. Personal samples were taken during daily work inside the greenhouse, or in the storage area beside the greenhouse when that place was the main workplace of the grower on the sampling day.

Temperature, relative humidity, and air velocity were measured with a multifunction instrument (Test 400; Testo, Lenzkirch, Germany) at the center of the greenhouse 1.5 m above the floor. CO₂ concentrations were measured with colorimetric detector tubes (Carbon Dioxide 100/a., 100–3,000 ppm; Draeger Sicherheitstechnik, Luebeck, Germany) with a manually operated pump (Accuro; Draeger Sicherheitstechnik). The details of dust sampling have been described elsewhere (17). In brief, airborne dust was collected on glass fiber filters placed in a holder with a constant airflow. Dust concentration was measured gravimetrically and related to air volume (lower detection...
formed with extracts from allergens present in the greenhouse area: to identify sensitization to workplace allergens, skin prick tests were performed. All samples were sent to a microbiology laboratory (Pegasus Labor, Dusseldorf, Germany) immediately after sampling and cultivated within the following 24 hours. The total concentration of airborne microorganisms was determined by the CAMNEA method (19) and quantified by inoculation of suitable dilutions of the extraction fluid on plates with selective media. After incubation, colony-forming units were counted and the concentration of microorganisms was expressed as colony-forming units per cubic meter (lower detection limit, 50 CFU/µL).

Lung Function
All subjects performed forced spirometry and reversibility testing before entering work according to standard techniques (20). Reference values were obtained from selected volunteers from the province of Barcelona (21). Current asthma was diagnosed when wheezing and/or asthma attacks within the past year were reported, FEV\textsubscript{1} was below 80% of reference, and the reversibility test was positive (greater than 12%) (22). All subjects reporting wheezing and/or asthma in the past year (n = 12), independent of their baseline lung function, were asked to undergo a bronchial provocation challenge at the workplace. First, FEV\textsubscript{1} was recorded every hour for eight hours on a nonexposure day to assess the variability of lung function. Second, FEV\textsubscript{1} was recorded again on a day when the subject was working inside the greenhouse, every half-hour for two hours, and hourly thereafter. Current occupational asthma was diagnosed when a sustained decrease ≥ 20% from baseline was observed in one or more successive measurements, in the absence of FEV\textsubscript{1} variability ≥ 10% on the control day, according to published guidelines (23, 24). Patients with a positive bronchial provocation challenge were asked to leave the greenhouse and continued to perform the scheduled spirometries outside to check their return to baseline values.

Skin Testing
Skin tests were performed at baseline by the prick method (25). Histamine phosphate (1/200 mg/mL) was used as the positive control and the diluent (glycerin, 50%) served as the negative control. The largest wheal diameter was measured 15 minutes after the allergen extract had been applied. A positive reaction was defined as a wheal ≥ 3 mm in the absence of reaction to the diluent and in the presence of a positive reaction to histamine phosphate. Atopy was defined by at least one positive reaction to eight common allergens (Dermatophagoides farinae and Dermatophagoides pteronyssinus, mixed trees, mixed grass, mixed graminae, Parietaria officinalis, cat hair, and dog hair). To identify sensitization to workplace allergens, skin prick tests were performed with extracts from allergens present in the greenhouse area: flowers (Gladiolus spp., Hyacinthus orientalis, Narcissus pseudonaranjus, Solidago canadensis, Chrysanthemum leucanthemum, and Helianthus annuus) and molds (Alternaria alternata, Aspergillus spp., Cladosporium herbarum, and Penicillium spp.) (Leti S. A., Barcelona, Spain, and Stallergènes, Antony, France).

Statistical Analysis
All data were entered into a database and analyzed with the SPSS (Chicago, IL) statistical software package. For categoric variables, results were expressed as absolute and relative frequencies, and for continuous variables as means and standard deviations or as medians and interquartile ranges, when the distribution was not normal. A value of half the lower detection limit was used for all measures of continuous variables showing a result below that value. The prevalence of occupational asthma was determined on the basis of the results of workplace bronchial challenge and the prevalence of sensitization to workplace allergens, taking into account the results of skin testing. Logistic regression modeling was used for the assessment of associations between variables, considering wheezing and sensitization to workplace allergens to be dependent variables. Age, sex, smoking, atopy, crop (flowers or ornamental plants), hours inside per day, application of pesticides by hand pump, frequency of pesticide use (categorized as < weekly or ⩾ weekly), and greenhouse characteristics (categorized as > median or ⩽ median) were considered independent variables. The results were expressed as crude and adjusted odds ratios (ORs), with 95% confidence intervals (CIs). Age and all variables showing an association with the outcome variable (p < 0.20) in the univariable analysis were included in the multivariable models, and the most parsimonious model that still explained the data was accepted as the final multivariable model. All statistical tests were two-tailed, and a p value equal to or less than 0.05 was reported as statistically significant.

RESULTS
Population
Thirty-nine flower and/or ornamental plant growers from the target population of 40 growers working inside greenhouses in Spain agreed to participate in the study (response rate, 97.5%). Skin testing was not accepted by one of the participants. Most growers cultivated 5–10 different flowers or ornamental plants, changing some of the species seasonally. Gladiolus spp., Chrysanthemum spp., Dianthus spp., Gerbera jamesonii, Rosa spp., and Geranium spp. were the most commonly cultivated greenhouse plants. A 7.7% prevalence of asthma attacks within the last year was reported by the enrolled growers, who were mainly middle-aged men. When the participants were compared with the subjects from the reference population of greenhouse flower and/or ornamental plants participating in the European Farmers’ Study but not included in the present study, only nonsignificant differences in age, sex, smoking, or prevalence of asthma attacks within the past year were found, suggesting that the studied sample was representative (Table 1).

Greenhouse Characteristics
Thirty-nine personal samples of dust, endotoxin, and microorganisms were obtained in the greenhouses (n = 34) or the storage areas beside the greenhouses (n = 5) (Table 2). Dust and endotoxin levels were low, sometimes failing to reach the detection limit of the technique, but in most cases airborne mold concentration was high, with a wide range of measurements. Recovered molds were mainly Cladosporium spp. (32 cases), Penicillium spp. (18 cases), Botrytis spp. (12 cases), Acremonium spp. (7 cases), Aspergillus spp. (7 cases), and Alternaria spp. (6 cases) (Figure 1).

Occupational Asthma and Sensitization to Workplace Allergens
Two subjects reporting wheezing and the three subjects who reported asthma attacks within the last year had lung function impairment confirming current asthma (12.8%). Workplace bronchial provocation challenge showed a pattern of occupational

<table>
<thead>
<tr>
<th>TABLE 1. GREENHOUSE GROWERS*: DESCRIPTIVE STATISTICS</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%)</td>
</tr>
<tr>
<td>Age, yr: mean (SD)</td>
</tr>
<tr>
<td>Sex (men), n (%)</td>
</tr>
<tr>
<td>Smoking (former or current), n (%)</td>
</tr>
<tr>
<td>Asthma attacks in past year, n (%)</td>
</tr>
<tr>
<td>FEV\textsubscript{1}, %pred: mean (SD)</td>
</tr>
<tr>
<td>FVC, %pred: mean (SD)</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Definition of abbreviation: NS = not significant.

\textsuperscript{* n = 33.}
asthma in three of these five growers, two with asthma attacks in the past year and one with wheezing but who was not diagnosed with asthma before the study (Table 3) (Figure 2). Bronchial challenge did not demonstrate a pattern of occupational asthma in the subjects reporting wheezing and/or asthma attacks within the past year who had normal lung function at baseline (n/H11011005). Thus, a 7.7% prevalence of current occupational asthma was found in the studied sample of greenhouse growers.

Sensitization to one or more workplace allergens was found in 13 growers (34.2%), to flowers in 8 cases (21.1%) (Chrysanthemum leucanthemum, 7; Solidago canadiensis, 4; Helianthus annuus, 4; Gladiolus spp., 2; Narcissus pseudonarcissus, 2; Hyacinthus orientalis, 1), and to molds in 7 cases (18.4%) (Aspergillus spp., 4; Alternaria spp., 4; Penicillium spp., 3; Cladosporium herbarum, 2). The three growers with occupational asthma were sensitized to various molds in one case (Aspergillus spp., Alternaria spp., Penicillium spp., and Cladosporium herbarum), to Gladiolus spp. in the second, and to Aspergillus spp. and various flowers the patient cultivated in the third (Gladiolus spp., Narcissus pseudonarcissus, Solidago canadiensis, Helianthus annuus, and Chrysanthemum leucanthemum). Current occupational asthma was then diagnosed in 3 of the 12 subjects sensitized to workplace allergens (23.1%).

Risk Factors for Wheezing and Sensitization to Workplace Allergens

Greenhouse characteristics and the concentration of air contaminants in the workplace were not significantly related to wheezing and sensitization to workplace allergens, and only a marginal association between wheezing and air velocity inside the greenhouse emerged from the present study (OR, 0.11; 95% CI, 0.03-0.43).

**TABLE 2. GREENHOUSE CHARACTERISTICS**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>Crop</td>
<td></td>
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<tr>
<td>Flowers, n (%)</td>
<td>25 (64.1)</td>
</tr>
<tr>
<td>Ornamental plants, n (%)</td>
<td>14 (35.9)</td>
</tr>
<tr>
<td>Hours per day of indoor work</td>
<td></td>
</tr>
<tr>
<td>Greenhouse, mean (SD)</td>
<td>4.4 (2.4)</td>
</tr>
<tr>
<td>Storage area, mean (SD)</td>
<td>4.9 (2.4)</td>
</tr>
<tr>
<td>Pesticide use</td>
<td></td>
</tr>
<tr>
<td>By hand pump, n (%)</td>
<td>8 (20.5)</td>
</tr>
<tr>
<td>≥ 1/wk, n (%)</td>
<td>30 (76.8)</td>
</tr>
<tr>
<td>Greenhouse characteristics†</td>
<td></td>
</tr>
<tr>
<td>Area, m²</td>
<td>1,140 (338–1,848)</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>24 (18–27)</td>
</tr>
<tr>
<td>Humidity, %</td>
<td>56 (46–64)</td>
</tr>
<tr>
<td>CO₂, ppm</td>
<td>500 (400–600)</td>
</tr>
<tr>
<td>Air velocity, m/s</td>
<td>0.31 (0.18–0.51)</td>
</tr>
<tr>
<td>Total dust, mg/m³</td>
<td>0.09 (0.08–0.21)</td>
</tr>
<tr>
<td>Endotoxin in total dust, ng/m³</td>
<td>0.32 (0.17–0.89)</td>
</tr>
<tr>
<td>Bacteria, CFU/m³</td>
<td>2,300 (770–5,200)</td>
</tr>
<tr>
<td>Molds, CFU/m³</td>
<td>5,000 (1,700–11,000)</td>
</tr>
</tbody>
</table>

* n = 39 growers.
† Expressed as median (interquartile range). In five cases personal samples of dust, endotoxin, and microorganisms were obtained in the storage area beside the greenhouse (see text for details).

**TABLE 3. OCCUPATIONAL ASTHMA AND SENSITIZATION**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current asthma†</td>
<td></td>
</tr>
<tr>
<td>Nonoccupational</td>
<td>2 (5.1)</td>
</tr>
<tr>
<td>Occupational</td>
<td>3 (7.7)</td>
</tr>
<tr>
<td>Sensitization‡</td>
<td></td>
</tr>
<tr>
<td>Atopy, n (%)</td>
<td>16 (42.1)</td>
</tr>
<tr>
<td>To workplace mold, n (%)</td>
<td>7 (18.4)</td>
</tr>
<tr>
<td>To workplace flowers, n (%)</td>
<td>8 (21.1)</td>
</tr>
<tr>
<td>To one or more workplace allergens, n (%)</td>
<td>13 (34.2)</td>
</tr>
</tbody>
</table>

* n = 39 growers.
† Current asthma confirmed by FEV₁/FVC < 80% of the reference and reversibility test > 12% from baseline in subjects reporting wheezing and/or asthma within the past year. Occupational asthma confirmed through bronchial provocation challenge in the workplace.
‡ Skin testing not accepted by one subject. Atopy defined as a positive reaction to at least one of eight common allergens and sensitization to workplace allergens as at least one positive reaction to workplace flowers and/or molds.

![Figure 1. Air contamination by molds inside greenhouses (n = 39 observations).](image)
Growers who did not report wheezing within the past year worked in more ventilated greenhouses (Table 4). Sensitization to workplace allergens was only associated with atopy (OR, 22.0; 95% CI, 3.65–133.51). In fact, subjects sensitized to workplace allergens were exposed to concentrations of molds in greenhouse air that were similar to those found in the greenhouses of nonsensitized subjects (Table 5).

DISCUSSION

To our knowledge, this population-based study is the first to examine the prevalence of occupational asthma in flower and/or ornamental plant growers working inside greenhouses. We found that nearly 8% of the growers had occupational asthma caused by workplace flowers and/or molds. Sensitization to
workplace allergens, however, was not related to greenhouse characteristics or to the concentration of indoor air contaminants.

The European Farmers’ Study reported a 3.2% asthma prevalence for crop farmers (8), a figure that was similar to that reported for the general European population (26). Analysis of specific crops in that study, however, showed an unusually high 5.1% prevalence of asthma for flower and/or ornamental plant growers, and work inside greenhouses emerged as an additional risk factor for asthma (8). In the present study, we found that the high prevalence of asthma in these growers is partly attributable to occupational asthma related to sensitization to flower allergens and/or molds present as contaminants in the air of the greenhouse. Our identification of occupational asthma in 7.7% of the greenhouse flower and/or ornamental plant growers agrees with the occasional published reports of individual cases of occupational asthma in flower handlers (27–32).

We found a 21% prevalence of sensitization to cultivated flowers in the studied greenhouse growers. Goldberg and co-workers (33), in a cross-sectional study of 75 flower growers, found sensitization to flower allergens in 52% of the subjects, a prevalence much higher than that found in a reference population. Of their flower growers, 15% abandoned the occupation because of unbearable symptoms, suggesting that their sensitization was clinically significant. In addition, we have also been able to demonstrate high greenhouse concentrations of molds, to which 18% of the subjects were sensitized, confirming the important role of these microorganisms in the respiratory symptoms of greenhouse flower and ornamental plant growers.

The low level of dust and endotoxin found inside the examined greenhouses did not suggest that these substances are clinically significant as triggers of the symptoms reported by the growers. Although exposure to endotoxin in grain handlers has been associated with respiratory symptoms (34), this effect is related to inhaled dose and does not appear when the workers are exposed to low concentrations (35). In spite of the low level of dust and endotoxin, poor ventilation emerged as a marginally significant risk factor for wheezing, perhaps because of the exposure to unidentified air contaminants that may reach irritant concentrations in less-ventilated greenhouses.

Sensitization to workplace allergens was surely related to the presence of flower allergens and molds in the greenhouses, but appeared in atopic subjects independently of the characteristics of the greenhouses where the plants were growing, as shown by the lack of association in our study between sensitization and the concentration of air contaminants in the workplace.

In our growers, we cannot rule out a possible allergenic role of cultivated flowers or ornamental plants to which skin testing was not performed. Similarly, we cannot discard allergy to *Tetranychus urticae*, the red spider mite, because we have not studied sensitization to this mite, which contaminates cultivated plants and which has been reported to be associated with the appearance of work-related symptoms (12–14, 36, 37). Astarita and coworkers (38) studied 46 symptomatic growers, some of whom worked inside greenhouses, and found that three-quarters were sensitized to this mite. The fact that in our study no positive bronchial provocation challenges were found in symptomatic growers who were not sensitized to the tested flowers and/or molds, however, is consistent with denying a clinically significant role of other flowers/ornamental plants or the red spider mite in the pathogenesis of occupational asthma in our growers.

In summary, the cultivation of flowers and/or ornamental plants inside greenhouses may cause occupational asthma in up to 8% of the growers through sensitization to flower allergens and workplace molds. Skin testing identifies sensitization.

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**TABLE 4. RISK FACTORS FOR WHEEZING**

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Present (n = 12)</th>
<th>Absent (n = 27)</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr: mean (SD)</td>
<td>45 (83.3)</td>
<td>50 (92.6)</td>
<td>0.95 (0.89–1.02)</td>
<td>0.92 (0.84–1.02)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>10 (83.3)</td>
<td>25 (92.6)</td>
<td>2.50 (0.31–20.27)</td>
<td>—</td>
</tr>
<tr>
<td>Smoking (current or former), n (%)</td>
<td>8 (66.7)</td>
<td>10 (37.0)</td>
<td>3.40 (0.81–14.23)</td>
<td>4.25 (0.81–22.31)</td>
</tr>
</tbody>
</table>

**Sensitization†**

- Atopy, n (%) 6 (50.0) 10 (38.5) 1.60 (0.40–6.36)
- Workplace allergens, n (%) 5 (41.7) 8 (30.8) 1.61 (0.40–6.63)
- Flowers, n (%) 7 (58.3) 18 (66.7) 0.70 (0.17–2.83)
- Hours per day in greenhouse, mean (SD) 5 (3) 4 (2) 1.17 (0.88–1.56)
- Hours per day in storage area, mean (SD) 4 (2) 5 (3) 0.89 (0.68–1.17)

**Pesticide use**

- By hand pump, n (%) 2 (16.7) 6 (22.2) 0.70 (0.12–4.10)
- ≥ 1/wk, n (%) 9 (75.0) 21 (77.8) 0.86 (0.17–4.21)

**Greenhouse characteristics‡**

- Area, m²: n (%) 8 (66.7) 11 (40.7) 2.91 (0.70–12.09) 3.85 (0.50–9.39)
- Temperature, °C: n (%) 5 (41.7) 13 (48.1) 0.77 (0.19–3.04)
- Humidity, %: n (%) 8 (66.7) 13 (48.1) 2.15 (0.52–8.89)
- CO₂, ppm: n (%) 6 (50.0) 9 (33.3) 2.00 (0.50–8.00)
- Air velocity, m/s: n (%) 4 (33.3) 14 (56.0) 0.39 (0.09–1.65) 0.09 (0.01–1.04)
- Endotoxin, ng/m³: n (%) 2 (28.6) 12 (54.5) 0.33 (0.05–2.10)
- Bacteria, CFU/m³: n (%) 5 (41.7) 14 (51.9) 0.66 (0.17–2.62)
- Molds, CFU/m³: n (%) 5 (41.7) 13 (48.1) 0.77 (0.19–3.04)

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*Definition of abbreviations: CI = confidence interval; OR = odds ratio.
† Age and all variables showing an association with the outcome variables (p < 0.20) in the univariable analysis included in the multivariable model.
‡ Skin testing not accepted by one subject. Atopy defined as a positive reaction to at least one of eight common allergens. Sensitization to workplace allergens defined as at least one positive reaction to flowers or molds.
§ Over the median, absolute and relative frequencies; logistic regression, > median versus ≤ median.
to these allergens in one-third of the growers cultivating flower and/or ornamental plants. The concentration of indoor allergens in one-third of the growers cultivating

**Acknowledgment:** The authors are grateful to R. Reinó, S. Alonso, S. Cabrera, and C. Rodríguez for field work, to M. de Sanpedro and F. Lopez for advice during the study, to J. Hartung for performance of the endotoxin measurements, and to M. E. Kerans for help in preparation of the manuscript.

**References**


