Neutrophilic Respiratory Tract Inflammation and Peripheral Blood Neutrophilia After Grain Sorghum Dust Extract Challenge*

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Study objective: To determine if inhalation of grain sorghum dust in the laboratory would cause neutrophilic upper and lower respiratory tract inflammation in human volunteers, as well as systemic signs of illness.

Design: Prospective.

Setting: University of Nebraska Medical Center.

Participants: Thirty normal volunteers.

Interventions: Inhalation challenge with 20 mL of a nebulized solution of filter-sterilized grain sorghum dust extract (GSDE). One group received prednisone, 20 mg for 2 days, prior to the challenge.

Measurements and results: Bronchoscopy with bronchoalveolar lavage (BAL) was performed 24 h after challenge, with samples collected as bronchial and alveolar fractions. Findings included visible signs of airways inflammation, quantified as the bronchitis index. The percentage of bronchial neutrophils was significantly increased in those challenged with GSDE vs the control solution, Hanks’ balanced salt solution (40.3±4.5% vs 14.3±5.1%, p<.01). Similar findings were seen in the alveolar fraction. Pretreatment with corticosteroids did not prevent the rise in neutrophils recovered by BAL. Peripheral blood neutrophils were also increased in volunteers challenged with the grain dust extract. To explain the increase in peripheral blood neutrophil counts, the capacity of the peripheral blood neutrophils to migrate in chemotaxis experiments was examined. The results demonstrate an increase in peripheral blood neutrophils and an increase in chemotactic responsiveness.

Conclusions: Inhalation challenge with a grain dust extract causes respiratory tract inflammation and a peripheral blood neutrophilia. One reason for this may be an increase in activated peripheral blood neutrophils.

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Key words: bronchitis; bronchoalveolar lavage; grain dust; inhalation challenge; nasal lavage; neutrophils

Inhalation of grain dust in the workplace causes acute bronchitis and nasal irritation. These are problems that decrease job satisfaction and cause individuals to change their occupation. It is also possible that repeated bouts of acute bronchitis lead to the chronic bronchitis seen in grain workers. Therefore, the acute respiratory tract symptoms seen after grain dust exposure merit further investigation.

The intensity of the bronchitis and rhinitis symptoms is related in part to the type of grain handled. Dust from grain sorghum causes particularly severe rhinitis and bronchitis, and is known to cause an influx of inflammatory cells into the lower respiratory tract of guinea pigs as demonstrated by lung lavage. Symptoms experienced after acute grain dust exposure suggest but do not prove that respiratory tract inflammation is present. The current investigation was designed to test the hypothesis that inhalation of grain sorghum dust extract (GSDE) causes upper and lower respiratory tract inflammation. To accomplish this, a method for challenge of normal volunteers with GSDE was developed. This challenge technique using a filter-sterilized extract administered by inhalation appears to be a safe and effective method of studying the acute effects of grain dust inhalation. Results derived from utilizing this technique demonstrate that the GSDE causes neutrophilic inflammation in both the upper and lower respiratory tract.

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For editorial comment see page 1194
METHODS

This research project was approved by the Institutional Review Board of the University of Nebraska Medical Center. Each volunteer gave written informed consent.

Preparation of GSDE

Settled grain sorghum dust from the air filter of a grain drying bin was collected on a farm. The GSDE was made as previously described. The filtered GSDE was cultured in the University of Nebraska Medical Center’s microbiology laboratory to rule out the presence of bacteria, fungi, and viruses.

Endotoxin is well known to be a component of grain dust. The amount of endotoxin in the GSDE was determined by a colorimetric limulus lysate method. The endotoxin level was found to be 1511 EU/mL of GSDE.

Settled grain dust typically contains spores from a variety of fungi, raising a concern about the presence of mycotoxins in extracts from grain dust. The GSDE was tested for the presence of mycotoxins courtesy of Dr. Allan Doster, Director of the Veterinary Diagnostic Center at the University of Nebraska-Lincoln. None of the following mycotoxins were present at detectable levels: aflatoxins B1, B2, G1, and G2, fumonisin, sterigmatocystin, citrinin, ochratoxin A, T-2 toxin, diacetoxyscirpenol, deoxynivalenol/vomitoxin, 3-acetyldeoxynivalenol, nivalenol, fusarenone-X, zearalenone, and zearalenol. The measurements were done using thin layer chromatography.

Subject Selection and Inhalation Challenge

Normal, nonsmoking volunteers were recruited for the study. All denied nasal disease, including allergic rhinitis and sinusitis. None had a history of asthma, chronic bronchitis, or other pulmonary diseases. All denied occupational exposure to fumes, organic dust, or inorganic dust. Individuals who described having an upper respiratory tract infection within the previous 6 weeks were excluded. Results of the screening examination of the nose and of the thorax were normal in all subjects accepted for the study. Chest radiographs were normal. The control group of subjects consisted of normal, nonsmoking individuals with negative exposure histories and normal results of physical examinations who received bronchoscopy with bronchoalveolar lavage (BAL) without inhalation challenge as part of a previous study.

Pulmonary function testing, consisting of spirometry with a single-breath diffusion capacity for carbon monoxide corrected for alveolar volume (Dco/VA: Medscience; Burlington, Miss) was performed prior to enrollment of each subject. All tests were performed in triplicate by trained pulmonary function laboratory staff and the highest value was reported. All had an FVC, FEV1, peak expiratory flow rate, and Dco that were in the normal range by Intermountain Thoracic Society standards. Nasal lavage was also performed during the screening assessment (method described below). Those who had greater than 60% nasal neutrophils on nasal lavage were not enrolled. The subjects were divided into groups as outlined below.

The GSDE and the Hanks’ balanced salt solution (HBSS) control solution were nebulized for inhalation using a compressor (Pulmoaid; DeVilbiss; Somerset, Pa) and a nebulizer (Airlife Misty-Neb; Baxter Healthcare Corporation; Valencia, Calif). The same equipment was used for all subjects. The subjects used masks that covered both the nose and the mouth. They rested in a sitting position during the challenge. The dose of administered solution was calculated assuming that 20 to 25% of the solution was deposited in the upper airway and that 10 to 15% of the solution used reached the lower respiratory tract. In order to deliver a dose of grain dust at the upper limits of what might be inhaled in a day spent working in a grain elevator, 24 mL of GSDE or the control HBSS was nebulized for each volunteer.

All volunteers had spirometry and a Dco performed at 0, +3, +5, +7, +9, and +11 h. At these time points, the volunteers were asked whether they were experiencing dyspnea, cough, chest tightness, malaise, headache, or stuffy nose. Temperature and pulse were measured. Blood was drawn for a CBC count at each of these time points and at +24 h.

A total of seven volunteers were studied to establish the safety of the grain dust extract challenge protocol. Challenges were performed with increasing doses of the GSDE, beginning with 3 mL of a 1:10,000 dilution. Final doses of GSDE administered by inhalation ranged from 3 to 12 mL of full-strength GSDE. Spirometry and a Dco were performed at 0, +1, +3, +5, +7, +9, and +11 h.

After the safety of the method had been established by testing the volunteers with increasing doses of GSDE, the remaining volunteers were divided into three groups as follows.

Group 1: Subjects were challenged with 24 mL of GSDE by inhalation. Nine men, aged 23 to 29 years, made up this group.

Group 2: Subjects were given prednisone, 20 mg three times a day, for 2 days prior to and on the day of the inhalation challenge with 24 mL GSDE. This group consisted of two women and four men, aged 23 to 35 years.

Group 3: Subjects were challenged with HBSS, used to prepare the GSDE. One woman and seven men, ranging in age from 22 to 46 years, were in this group.

Bronchoscopy and BAL

Bronchoscopy and BAL were performed at +24 h to assess the lower respiratory tract for signs of inflammation. This time point was chosen for practical convenience and because previous studies demonstrated the presence of lower respiratory tract inflammation 24 h after a stimulus. Bronchoscopy was performed transorally using a flexible fiberoptic bronchoscope (Olympus Type 1T-10 or P-10; Olympus Corporation of America; Aurora, Colo). All bronchoscopies were done by the same individual. Subjects received an aerosol that contained 3 mL of a 1% lidocaine solution and 2.5 mL of a 5% albuterol solution before bronchoscopy. The airways were further anesthetized as needed with 2% and 1% lidocaine solutions during the bronchoscopy. Subjects were given atropine prior to the bronchoscopy, and sedated as required for comfort using intravenous meperidine and midazolam.

Scoring for visible signs of bronchitis was done during the bronchoscopy as previously described. Points were assigned for erythema, edema, friability, and secretions (0=normal, 1=mild, 2=moderate, and 3=severe). Each lobe of the lung and the lingula were scored separately, and the total number of points was reported as the bronchitis index.

The BAL was done in three lobes of the lung by placing the bronchoscope in a wedged position in a subsegmental bronchus, and then consecutively instilling and immediately aspirating by gentle suctioning of each of the five 20-mL aliquots of saline solution. Lavage material returned from the first 20-mL aliquots represented largely bronchial material and was pooled for analysis. The returns from the subsequent aliquots of saline solution were pooled and analyzed as the alveolar fraction.

The BAL specimens were processed for quantification of cell differentials. Total cell counts were done on the bronchial and alveolar fractions before further processing, using a hemacytometer. Mucus was removed from the BAL specimens by nylon mesh filtration. Cytocentrifuge preparations were made from the bronchial and alveolar cells (Shandon CytoSpin 2; Astmoor, England) and stained (with Diff-Quik; American Scientific Products; McGaw Park, Ill). Differential cell counts were performed by having two observers count 200 cells on each cytocentrifuge preparation and reporting the mean of the two counts. Data from normal control subjects studied previously provided reference values for the bronchitis index and BAL cell counts and differentials.

1426 Occupational and Environmental Lung Disease

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Lavage
Nasal
the volunteer
aliquots
5-mL
respiratory
tract.
repeated
24
so
chosen
percent
HBSS
greater
lute
Figure 1.
I
lavage, initially
performed
as a screening
procedure, was
repeated
24 h after
challenge. The
postchallenge
time point was
chosen so that
results could be
 correlated
with findings in
the lower
respiratory
tract. Nasal
lavage was
done by
serially
instilling three,
5-mL
aliquots
of
sterile
saline
solution
into
each
nostril
and
having
the
volunteer
forcefully
expel
the
fluid
into
a
basin.
The
fluid
was
strained
to
remove
mucus
and
processed
as
described
for
the
BAL
specimens.

Neutrophil Chemotaxis

To
determine
if
peripheral
blood
neutrophils
from
the
study
subjects
were
primed
and
therefore
more
responsive
to
a
standard
chemoattractant,
following
challenge
with
GSDE
vs
HBSS,
neutrophil
chemotaxis
was
performed
using
peripheral
blood
neutrophils
from
study
subjects.
These
neutrophils
were
collected
at
0, +7,
and
+24 h,
and
chemotaxis
was
done
using
a
modified
blindwell
 technique.1
Potential
chemoattractants,
placed
in
the
lower
wells
of
the
chamber
(Neuro
Probe;
Cabin
John,
Md)
included
zymosan-
activated
serum
and
GSDE,
both
known
to
cause
neutrophil
chemotaxis.3
The
lower
wells
of
the
chamber
were
covered
by
a
3-µm
membrane
filter
(Nucleopore;
Neuro
Probe).
The
volunteers’
leukocytes,
consisting
mostly
of
neutrophils,
were
isolated
by
dextran
sedimentation,
suspended
at
3
million/mL,
and
placed
in
the
upper
wells
of
the
chemotaxis
chambers.
After
a
20-min
incubation
period,
the
membranes
were
removed
and
stained
with
a
modified
Wright’s
stain
(Lawrence;
Scientific;
Fair
Lawn,
NJ),
and
mounted
on
glass
slides.
Chemotactic
activity
was
quantified
by
counting
the
cells
migrating
through
each
membrane
using
the
X40
objective
of
a
light
microscope
attached
to
an
image
analyzer
(Opti-
tomax
5
Image
Analyzer;
Analytic
Measuring
Systems;
Cambridge,
England).
Each
sample
was
assayed
in
sextuplicate
and
10
high-
powered
fields
were
counted
per
assay.

Statistical
Analysis

Results
from
the
group
of
subjects
challenged
with
GSDE
alone
were
compared
with
those
treated
with
prednisone
and
challenged
with
GSDE,
as
well
as
with
those
who
received
the
negative
control
substance
HBSS.
These
results
were
also
compared
with
those
from
unchallenged
control
subjects
described
previously.5
All
comparisons
were
done
using
repeated
measures
analysis
of
variance.
A
level
of
less
than
or
equal
to
0.05
was
accepted
as
statistically
significant.
Results
are
reported
as
mean
values
±
SEM.

RESULTS

Bronchitis
Index

The
visually
assessed
mean
bronchitis
index
in
the
GSDE-challenged
subjects
(group
1)
was
found
to
be
significantly
increased
when
compared
with
those
challenged
with
HBSS
(7.9±0.6
vs
4.0±0.7,
p<.001).
The
mean
bronchitis
index
of
the
corticosteroid-
treated,
GSDE-challenged
subjects
(group
2)
was
significantly
less
than
in
those
who
only
received
GSDE
(4.7±1.5
vs
7.9±6,
p<.05)
and
did
not
differ
from
the
HBSS-challenged
subjects.
Those
who
received
HBSS
had
a
bronchitis
index
that
was
slightly
greater
than
that
of
the
normal
control
subjects
(2.6±0.8),
but
this
difference
did
not
reach
statistical
significance.

Cell
Populations
in
the
Bronchial
Lavage
Sample

Bronchial
neutrophils,
assessed
by
both
percentage
and
number
per
milliliter,
were
increased
significantly
above
HBSS-challenged
control
values
in
the
GSDE-
challenged
subjects
(Fig
1).
Individuals
challenged
with
GSDE
had
a
significantly
higher
percentage
of
bronchial
neutrophils
than
those
challenged
with
HBSS
(40.3±4.5% vs
14.3±5.1%,
p<.01).
Glucocorticoid

Nasal
Lavage

Nasal
lavage,
initially
performed
as
a
screening
procedure,
was
repeated
24
h
after
challenge. The
postchallenge
time
point
was
chosen
so
that
results
could
be
 correlated
with
findings
in
the
lower
respiratory
tract.
Nasal
lavage
was
done
by
serially
instilling
three,
5-mL
aliquots
of
sterile
saline
solution
into
each
nostril
and
having
the
volunteer
forcefully
expel
the
fluid
into
a
basin.
The
fluid
was
strained
to
remove
mucus
and
processed
as
described
for
the
BAL
specimens.
pretreatment had no effect. Neutrophils were increased slightly in the HBSS-challenged group when compared with unchallenged control subjects, but statistical significance was not reached. Bronchial neutrophils per milliliter of lavage fluid were also significantly higher than in those challenged with GSDE than in those who received HBSS (0.15 × 10⁶ polymorphonuclear leukocytes [PMN] per milliliter ± 0.03 vs 0.05 × 10⁶ ± 0.02, p < 0.01). Again, glucocorticoid treatment did not decrease neutrophil number.

Other inflammatory cell types quantified in the bronchial sample included lymphocytes, eosinophils, and macrophages (Table 1). Eosinophil and lymphocyte populations in the bronchial fraction of the BAL fluid were not significantly changed in number after challenge. When bronchial macrophage counts were assessed, it was found that the percent bronchial macrophages was lower in the GSDE-challenged groups than in those challenged with HBSS, which is likely a reflection of the increased percentage of neutrophils in those who received GSDE. However, there was no significant difference between groups 1 and 2 vs group 3 in the absolute numbers of these cells.

Total cell counts per milliliter of lavage fluid were not significantly greater in those challenged with GSDE than in those challenged with HBSS. In group 2 subjects, treated with corticosteroids and GSDE challenged, macrophages were the only type of cells that were significantly increased compared with the HBSS control group. The HBSS-challenged group did not have total cell counts greater than those of unchallenged controls.

Cell Populations in the Alveolar Lavage Sample

Alveolar neutrophils were assessed by both percentage and alveolar neutrophils per milliliter of lavage fluid and were increased following GSDE challenge (Fig 2). Those who received the GSDE challenge alone had a significantly greater percentage of neutrophils than those challenged with HBSS (47 ± 7.4% vs. 9.7 ± 4.9%, p ≤ 0.01). Neutrophils per milliliter of lavage fluid were also significantly increased in the

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**Table 1—Total Cells, Macrophages, Eosinophils, and Lymphocytes in the Bronchial Sample***

<table>
<thead>
<tr>
<th>% Total Cells</th>
<th>Cells × 10⁶/mL BAL Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1</td>
</tr>
<tr>
<td>Total cells</td>
<td>--</td>
</tr>
<tr>
<td>Macrophages</td>
<td>40.3 ± 4.3 ³</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>2.1 ± 1.8</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>2.5 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.38 ± 0.05</td>
</tr>
</tbody>
</table>

*Values are stated as the standard error of the mean.
³Group 1 vs group 3 and vs control, both p ≤ 0.001.
²Group 2 vs group 3 and vs control, both p ≤ 0.05.
¹Group 3 vs group 2 and vs control, both p ≤ 0.05.

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**Figure 2.** Alveolar neutrophils in subjects challenged by inhalation of GSDE ± corticosteroids or HBSS. As shown in panel A (top), the percent alveolar neutrophils for both groups 1 and 2 were significantly higher than for the group that received only HBSS (p values for both comparisons, < 0.01). Panel B (bottom) shows absolute alveolar neutrophils, which again were significantly greater in both groups challenged with GSDE (p values < 0.01 and 0.05, respectively).

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GSDE-challenged group (0.26×10⁶ PMNs/per milliliter ±0.14 vs 0.02±0.01, p=0.01). Prednisone therapy did not lower the neutrophil numbers. Alveolar neutrophils were slightly higher in those challenged only with HBSS than in the unchallenged subjects, but this did not reach significance.

Total cells per milliliter of lavage fluid returned were significantly increased in both groups challenged with GSDE, compared with those who received HBSS and the control subjects (Table 2). The inflammatory cells quantified, besides neutrophils, included macrophages, lymphocytes, and eosinophils. Again, numbers of lymphocytes and eosinophils, assessed by both percentage and numbers of cells per milliliter, were within the normal range in all subjects and did not change after GSDE inhalation challenge. The percentage alveolar macrophages was significantly lower in groups 1 and 2, compared with those challenged with HBSS, as expected given the elevation in percentage neutrophils in those groups. However, alveolar macrophages per milliliter of lavage fluid were not significantly increased above the HBSS-challenged group in those who received GSDE alone or GSDE and corticosteroids.

**Nasal Lavage**

When baseline and postchallenge values were compared, nasal neutrophils were increased as percentage of recovered cells in the GSDE-challenged group (22.0±5.2% vs 51.8±12.7%, p<0.05), but not in the HBSS control group (24.3±6.5% vs 30.9±7.3%, NS). The group given GSDE and corticosteroids did not have a significant rise in percent nasal neutrophils (32.2±9.4% vs 47.3±11.2%, NS). However, differences between the groups in percent nasal neutrophils did not reach statistical significance.

The absolute numbers of nasal neutrophils at baseline were also compared with the values after inhalation challenge. An increase was seen the GSDE-challenged group, but this was not significant (0.02×10⁶ PMNs per milliliter ±0.01 vs 0.04±0.02, NS). Those challenged with GSDE and treated with corticosteroids and those who were challenged with HBSS did not have an increase in the numbers of nasal neutrophils per milliliter of nasal lavage fluid (data not shown).

**Peripheral Blood Neutrophil Counts**

WBCs were in the normal range for all volunteers at baseline. The WBCs were at the upper limits of normal at baseline in group 2, which likely represents corticosteroid effect. At the next time point, +3 h, the mean WBCs in group 1 and group 2 were significantly elevated compared with baseline, and both were significantly greater than those of group 3 (10.5

![Figure 3. Absolute peripheral blood neutrophils after challenge with GSDE±corticosteroid or HBSS. The absolute numbers of neutrophils were significantly elevated in groups 1 and 2 compared with group 3 at +3, +5, +7, and +9 h (p<0.001, all comparisons). At +5, +7, and +9 h, those who received corticosteroid with the GSDE had significantly more WBCs and PMNs than those who received GSDE alone (p<0.01, all comparisons).](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21724/ on 06/26/2017)
Table 3—Pulmonary Function Before and After Challenge With GSDE ± Corticosteroids or With HBSS

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>+30 min</td>
<td>+7 h</td>
</tr>
<tr>
<td>FVC</td>
<td>101.1±4.4</td>
<td>97.1±4.4*</td>
<td>99.0±3.8</td>
</tr>
<tr>
<td></td>
<td>102.6±2.9</td>
<td>100.8±3.2</td>
<td>102.1±2.9</td>
</tr>
<tr>
<td>FEF_{25-75}</td>
<td>100.2±7.8</td>
<td>101.1±2.8</td>
<td>100.3±3.2</td>
</tr>
<tr>
<td></td>
<td>100.2±4.0</td>
<td>100.2±3.8</td>
<td>100.2±3.2</td>
</tr>
<tr>
<td>FEF_{50}</td>
<td>99.8±1.5</td>
<td>95.0±3.8</td>
<td>97.3±3.7</td>
</tr>
<tr>
<td></td>
<td>97.3±3.7</td>
<td>97.3±3.7</td>
<td>97.3±3.7</td>
</tr>
<tr>
<td>Dco</td>
<td>91.6±3.6</td>
<td>96.6±4.1</td>
<td>97.9±2.5*</td>
</tr>
<tr>
<td></td>
<td>94.8±2.6</td>
<td>95.0±3.8</td>
<td>99.5±4.7</td>
</tr>
</tbody>
</table>

*Group 1 vs baseline. FVC, p≤0.001. FEF_{25-75}, p≤0.05. FEF_{50}, p≤0.01.

1 Group 2 vs baseline. Dco, p≤0.05.

All groups had significant and elevate responses to GSDE in peripheral blood. These increases were lower in group 3 compared to group 1 and 2. The increase in group 1 was significantly higher than in group 2, suggesting that corticosteroid decreased the response (p<0.01).

WBC×10^3/μL×1.4 and 12.5+1.1 vs 5.9+0.3, p<0.01 (for both comparisons). The WBCs remained elevated in both groups 1 and 2 through the +9 h time point, but was approaching baseline in all groups at +24 h.

The absolute number of neutrophils increased in parallel to the WBC count and accounted for the rise in that value at each sampling time (Fig 3). Other peripheral blood leukocytes did not change. Again, all groups were within the normal range at 0 h (1.0 to 7.5 K/cm³). The peak value was noted at +9 h for group 1, which was significantly higher than the value for group 3 (9.1 K/cm³±0.5 vs 3.3±0.2, p<0.001). Group 2 also had markedly elevated absolute neutrophil counts in peripheral blood. All had returned to the normal range at +24 h.

Chemotaxis of Peripheral Blood Neutrophils

The ability of the peripheral blood neutrophils of each subject to migrate toward the known chemoattractant GSDE was assessed at baseline prior to the challenge, and these values were compared with those obtained in the same individual 7 h and 24 h later (Fig 4). All GSDE-exposed subjects had increased chemotaxis to GSDE after 7 h following response. However, the increase was less in GSDE-challenged and glucocorticoid-treated subjects than those who received GSDE alone (92.9%±3.8 vs 124%±5.3 vs 153%±4.9, p<0.01). Values in all subjects challenged with GSDE had returned to baseline at +24 h. The HBSS control-challenged subjects had no significant change in neutrophil migration at +7 h or +24 h. Neutrophil chemotaxis to zymosan-activated serum gave similar findings with the exception that the differences between group 1 and group 2 at +7 h were not statistically significant (data not shown).

Pulmonary Function Tests

The mean FEF_{25-75}, FVC, FEF_{50}/FVC, and Dco/VA values were within the normal range for each group at baseline and at all subsequent time points (Table 3). All values are reported as percent predicted. However, the GSDE-challenged subjects of group 1 had significant decreases in the FVC, the FEF_{50}, and the FEF_{50}/FVC ratio at +30 min. The diffusing capacity (Dco) did not fall in those challenged with GSDE.

In contrast, the group that received corticosteroids with the GSDE challenge had no significant changes in their mean FEF_{25-75}, FEF_{50}/FVC ratio, or Dco at +30 min. There was a decrease in the mean FVC, noted only at +3 h. Those challenged with HBSS alone only had a slight but significant decrease in the FVC, seen at +30 min.

Small changes in FEF_{25-75} and FEF_{50} were noted as well. There were statistically significant decreases in FEF_{25-75} and FEF_{50} at +30 min for members of all groups, but no significant differences between groups.
Table 4—Complaints After Challenge With GSDE ± Corticosteroids or With HBSS

<table>
<thead>
<tr>
<th>Fever</th>
<th>Cough</th>
<th>Chest Tightness</th>
<th>Myalgias</th>
<th>Headache</th>
<th>Stuffy Nose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (n=8)</td>
<td>2 (25%)</td>
<td>3 3 4 2 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 2 (n=6)</td>
<td>0 0 3 3 1 0 2</td>
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<tr>
<td>Group 3 (n=9)</td>
<td>0 1 0 0 0 0 0</td>
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*Seven of eight had complaints.
Five of six had complaints.
One of nine had complaints.

Symptoms and Physical Examination

A total of 26 complications of the inhalation challenge were noted, consisting of fever, cough, chest tightness, myalgias, headache, and stuffy nose (Table 4). There were 16 complaints noted in those who received GSDE alone, 9 complaints in those who were given corticosteroids and the GSDE challenge, and 1 complaint in the HBSS group.

Discussion

In this study, inhalation challenge with a sterilized extract of grain sorghum dust induced signs and symptoms of respiratory tract inflammation. This included visible evidence of inflammation and the presence of increased numbers of neutrophils recovered by BAL. There was a mild, transient fall in FVC, FEV₁, and FEV₁/FVC. Systemic effects included the presence of increased numbers of neutrophils in the peripheral blood. These neutrophils also demonstrated increased chemotaxis. There was also evidence for the presence of neutrophilic nasal inflammation after GSDE challenge. It is likely that the effect seen in the nose would have been more pronounced at an earlier time point. The effects seen were partially blocked by treatment with corticosteroids. It is of note that the numbers of neutrophils in the lower respiratory tract did not change with this therapy.

This simple model of grain dust exposure in the workplace is useful for studying the effects of grain dust on the human respiratory tract. Limitations of the model include the fact that material in the grain dust which could contribute to inflammation was removed in the process of preparing the extract. Filter sterilization of the grain dust extract removed infectious agents that could have caused additional lower respiratory tract inflammation. However, this measure was necessary to assure the safety of the study subjects. The fact that the model may be considered to be safe for use in normal volunteers contributes to its potential for future applications.

A previous study by doPico et al has shown that pulmonary and systemic symptoms can be elicited by challenge with a grain dust extract delivered by nebulizer. A recent study by Clapp et al demonstrated that corn dust extract inhalation challenge also results in neutrophilic lower respiratory tract inflammation in grain workers, as well as release of mediators of inflammation. However, cellular changes after and visible effects of grain dust extract challenge have not been completely characterized. Our study demonstrates that directly visible signs of acute bronchitis and a neutrophilic upper and lower respiratory tract inflammation can be induced by inhalation of a grain dust extract. Hanks’ balanced salt solution, the material used to prepare the GSDE, induced a mild neutrophil influx. However, the evidence suggests that most of the changes seen were caused by the GSDE. Using the technique of separate analysis of the first returned aliquot of lavage fluid to separate the sample enriched with bronchial material from that enriched with alveolar material, we showed that the increase in numbers of neutrophils occurs both in the bronchi and in the distal structures. This finding is likely related in part to the size of the particles generated by the nebulizer and their deposition in the respiratory tract. The neutrophilic lower respiratory tract inflammation was associated with respiratory and systemic symptoms in a greater number of the subjects challenged with GSDE than with HBSS challenge. These findings suggest that this model is also useful for evaluating the symptoms experienced after grain dust exposure.

Complaints of nasal stuffiness are common after grain dust exposure. Some individuals in the two groups exposed to GSDE, but not to HBSS, had this complaint. When inflammation in the nose was assessed by counting the numbers of neutrophils, it was found that there was a smaller response than was seen in the lower respiratory tract. It is possible that a greater effect would have been noted if the nasal lavage had been performed earlier.

The systemic effects of GSDE challenge quantified in this study consisted of fever and an increase in the numbers of peripheral blood neutrophils, as well as their ability to migrate toward a stimulus. It is possible that challenge with GSDE causes release of the mediators of inflammation from the lower respiratory tract, such as tumor necrosis factor and interleukin-1, which are known to cause fever and a leukocytosis.

It is uncertain which component(s) of GSDE causes the findings described above. It is very likely that endotoxin plays an important role in causing the effects noted. However, previous studies have shown that GSDE causes more neutrophil recruitment than endotoxin alone in amounts equal to those present in the GSDE. This is evidence that human challenge with cotton bract causes lower respiratory tract inflammation.
tion that does not correlate well with endotoxin levels in the bract. There is also evidence that decreasing the amount of endotoxin in GSDE does not cause a proportionate decrease in the ability to attract neutrophils in vitro. It is likely that endotoxin and other substances found in grain dust act together to cause respiratory tract inflammation.

Corticosteroids are used to treat airway disease where neutrophilic inflammation is present. Treatment with inhaled corticosteroids decreases the number of neutrophils present in the lower respiratory tract of patients with bronchitis. This is not a surprising observation, as corticosteroids are known to decrease production of leukotriene B4, tumor necrosis factor alpha, and interleukin-1, which are all involved in neutrophil recruitment. It is not clear why corticosteroids did not decrease the influx of neutrophils into the respiratory tract of normal human subjects challenged with GSDE. Perhaps the release of tumor necrosis factor and interleukin-1 by macrophages in the lower respiratory tract, which is not blocked by corticosteroids, indirectly caused the neutrophil influx. Alternatively, neutrophils may have been recruited directly, a mechanism for which there is evidence in in vitro studies.

There were differences between those who received the GSDE alone and those also given corticosteroids. These included the lower bronchitis index in the corticosteroid-treated group, suggesting that inflammation was decreased when measured by means other than neutrophil influx. The decrease in peripheral blood neutrophil chemotaxis may indicate the presence of corticosteroid effect, as may the fact that there was no decrease in the FEV1/FVC ratio in this group, as well as fewer symptoms. Further work is needed to determine if steroids exert a clinically important anti-inflammatory effect in the setting of grain dust exposure.

In summary, inhalation challenge with GSDE caused an influx of neutrophils into the respiratory tract. The effect on neutrophil migration was more pronounced in the lung than in the nose at the time point studied and was not blocked by treatment with corticosteroids. There was, however, some evidence for an anti-inflammatory effect of corticosteroids after inhalation of GSDE. We propose that this inhalation challenge model be used together with bronchoscopy with BAL and nasal lavage as a means of studying occupational respiratory tract inflammation. It is a simple, safe method suitable for testing the efficacy of pharmacologic agents to block the appearance of symptoms, an influx of neutrophils, and potentially the release of mediators of inflammation after organic dust exposure.

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