Myiasis Due to Hypoderma lineatum Infection Mimicking the Hypereosinophilic Syndrome

JOHN STARR, MD; JOHN H. PRUETT, PHD; JOHN W. YUNGINGER, MD; AND GERALD J. GLEICH, MD

Myiasis is the infestation of live humans with larvae of Diptera (true flies). This report describes a protracted illness caused by infestation with Hypoderma lineatum, resembling the hypereosinophilic syndrome. A 35-year-old man had a 9-month multisystemic illness with pronounced eosinophilia, pleuritis, pericarditis, and myositis. Treatments including glucocorticoids did not alter the disease. Diagnostic studies included computed tomography, 2-dimensional echocardiography, leukocyte count, surgical biopsy of skin and muscle, blood immunoglobulin levels, and blood chemistry. Myiasis was recognized when a worm emerged from the patient’s skin; after a second worm emerged, the patient’s symptoms disappeared rapidly.

Myiasis is the infestation of live human or other vertebrate animals with larvae of Diptera (true flies). The ancient Romans knew about the serious economic effects of myiasis caused by cattle grubs on skins intended for leather manufacture. Fifty species of Diptera produce myiasis in humans, and most are unimportant; however, several cause serious lesions. For example, larvae from Hypoderma lineatum are hatched from eggs laid on hair, can penetrate unbroken skin through the follicles, feed on internal tissues, and migrate in the first instar stage. During such infestations, massive blood and tissue eosinophilia and a condition resembling the hypereosinophilic syndrome can develop.

We describe a 35-year-old man who had a protracted multisystemic illness with pronounced eosinophilia, pleuritis, pericarditis, and myositis. When a maggot emerged from his skin, myiasis was diagnosed. This malady should be considered in the differential diagnosis of several syndromes associated with eosinophilia, including the hypereosinophilic syndrome.

REPORT OF A CASE

A 35-year-old cattle rancher and oil field worker in Texas was healthy until March 1995, when he noted gradual onset of bilateral arm and shoulder pain, as well as back and chest discomfort. Additionally, cough and chest tightness occurred, but he had no wheezing. Symptoms persisted after glucocorticoid and doxycycline treatment. In June 1995, right upper quadrant abdominal discomfort and nausea developed. Further treatment included H₂ blockers and a proton pump inhibitor. Findings on upper gastrointestinal tract radiography, gallbladder ultrasonography, and esophagoduodenoscopy were normal; Trichinella antibody and stool test results for parasites were negative. During August and September 1995, the patient developed night sweats, chills, rigors, and intermittent fever (temperature up to 39°C). Severe diffuse myalgia occurred, and pleural and pericardial rubs were heard. Computed tomography (CT) of the chest disclosed bilateral pleural effusions and a moderate-sized pericardial effusion. A complete blood cell count yielded 16.7 x 10⁹/L leukocytes (reference ranges shown parenthetically) (3.5-10.5 x 10⁹/L) with 48% eosinophils. At that time, swelling and tenderness of the patient’s left testicle developed. Still disease was diag-

For personal use. Mass reproduce only with permission from Mayo Clinic Proceedings.
Myiasis and Hypereosinophilic Syndrome

Figure 1. Second instar stage of *Hypoderma lineatum*. The white larva was about 1 cm long and 0.15 cm in diameter. It emerged from the patient's skin on December 18, 1995. Morphologic identification was based on distinctive features in the spicular plate (the breathing apparatus on the posterior end) to distinguish between the species and larval stage.

nosed and treated with aspirin. Symptoms persisted, and he was hospitalized. His leukocyte count was 27.4 x 10^9/L, with 32% eosinophils. Repeated leukocyte counts ranged from 16.8 to 30.0 x 10^9/L, with the absolute eosinophil numbers varying from 8.0 to 22.6 x 10^9/L (0.05-0.5 x 10^9/L). Results of a rheumatoid factor latex test were elevated at 80.2 IU/mL (0-19 IU/mL); the alkaline phosphatase level was slightly elevated. A second chest CT scan showed small pleural effusions and a tiny pericardial effusion. Abnormal fluid accumulation occurred in the right upper posterior chest tissue planes; later, pronounced swelling developed in this area. In early October 1995, surgical biopsy of the latissimus dorsi and overlying skin showed severe inflammation and eosinophilic infiltration; no parasites were detected. Nonsteroidal anti-inflammatory drugs provided no improvement. In mid-October 1995, the patient's IgE was 1547 kU/L (0-240 kU/L), IgG was 1480 mg/dL (700-1500 mg/dL), IgA was 317 mg/dL (60-400 mg/dL), IgM was 311 mg/dL (60-300 mg/dL), and the erythrocyte sedimentation rate was 30 mm/1 h (0-20 mm/1 h). Late in October, small pruritic nodules appeared on the back of the patient's legs and around his waist, persisting for 4 to 7 days. A biopsy specimen from a right posterior thigh lesion disclosed perivascular infiltration with eosinophils and mononuclear cells. After the overall clinical picture was reviewed, the hypereosinophilic syndrome was diagnosed, and prednisone was initiated at 60 mg/d. A month later, while receiving prednisone every other day, the patient continued to have urticaria, modest eosinophilia (12%), and a leukocyte count of 10.0 x 10^9/L. Two months later, on December 18, 1995, a new skin lesion developed on the patient's lower abdominal area. When he rubbed this lesion, a white worm (about 1 cm long) emerged (Figure 1). This larva was *H lineatum*, second instar stage. The prednisone dosage was rapidly reduced and discontinued. Two weeks later, a second larva exited from a skin lesion on the patient's back. The patient's symptoms disappeared rapidly; 3 weeks later he had normal numbers of eosinophils.

**Histological Analyses**

Surgical biopsy specimens were analyzed for eosinophil granule major basic protein (MBP) by using a previously described indirect immunofluorescence assay. Extensive extracellular MBP deposition was noted between muscle bundles and in disrupted fascia with few intact eosinophils (Figure 2). Staining with hematoxylin-eosin showed extensive perivascular and interstitial inflammation with few eosinophils; the muscle fibers were well preserved. Serial sections stained with normal rabbit IgG were negative.

**Detection of IgE and IgG Antibodies to *H lineatum***

For detection of IgE antibodies, an extract of *H lineatum* first instar larvae (HL1; 104 mg/mL) was bound to paper disks and allowed to react with 0.050 mL of serum. After washing, IgE antibodies were detected with radiolabeled anti-IgE. To measure IgG antibodies, HL1 bound to microtiter plates was reacted with diluted serum. Bound IgG antibody was detected by the enzyme-linked immunosorbent assay (ELISA). The elevated levels of IgE and IgG antibodies for HL1 antigens on February 22 (about 2 months after worm emergence) and in June 1996 are shown in Table 1. Among the IgG subclasses, antibodies of the IgG4 subclass dominated but decreased between February and June.

**Western Blot Analyses for *H lineatum* Antigens**

Electrophoresis with use of 12% polyacrylamide gels with sodium dodecyl sulfate separated 8 to 10 protein bands in HL1 (Figure 3, A). After being blotted onto nitrocellulose, the proteins were reacted with the patient's serum and developed with either radiolabeled anti-IgE or staphylococcal protein A. These immunoblots revealed IgE and IgG antibodies for various HL1 proteins (Figure 3, B and C), indicating a heterogeneous antibody response.

**DISCUSSION**

*H lineatum*, the common cattle grub or warble botfly, and *Hypoderma bovis*, the northern cattle grub, are obligate insect parasites of cattle. Losses from these parasites are estimated at $66 million per year, primarily as a result of damaged hides. In January or February, depending on the latitude, the adult *H lineatum* female fly, the only species native to Texas, attaches eggs at the base of host hairs with a specialized ovipositor. Eggs hatch in approximately 3 to
In February through April, the newly hatched first instar larvae penetrate the skin via the hair follicles and migrate during April through June to connective tissue sites around the diaphragm and esophagus. The larva resides there approximately 4 months, then (in September or October) migrates to the subcutaneous tissues of the back. During October through December, each larva digests a breathing hole and resides in a furuncle (warble) for about 2 months, where it molts twice. The larva exits the host, pupates in loose soil, and develops to the adult fly stage. Remarkably, in our patient, the larval survival time was consistent with the cow host, even though the human is an aberrant host.

Although patients with myiasis due to *H. lineatum* or *H. bovis* have been described previously, the incidence of this disease is difficult to assess because most cases are probably never reported. Several cases of myiasis have been documented among farmworkers in France. Immuno-electrophoresis and ELISA can serologically diagnose human hypodermiasis; sera from patients with other parasitosis showed no response to *H. lineatum* antigens. These tests use soluble proteolytic enzymes extracted from the first instar larva as antigens. The larval collagenase molecule contains the most commonly shared epitopes between the soluble antigens of *H. lineatum* and *H. bovis*. The *H. lineatum* collagenase (hypodermin) has been thoroughly studied. An ELISA diagnostic test that uses antibodies specific for hypodermin should be diagnostic for human hypodermiasis caused by either species.

Our patient's immune response included leukocytosis, eosinophilia, elevated total IgE level, degranulation of tissue eosinophils, and increased IgE and IgG antibodies specific for the HL1 antigens. Of note, his IgG titer decreased between February and June 1996; an increasing titer should indicate ongoing infection. Interestingly, the IgG response was mainly IgG4, the predominant blocking antibody in chronic helminth infections. Prolonged antigenic stimulation, eg, allergy immunotherapy and allergic bronchopulmonary aspergillosis, is probably important in stimulating IgG4 production.

Several features of our patient's illness suggested the idiopathic hypereosinophilic syndrome. This syndrome covers a heterogeneous patient group, with an idiopathic (no apparent causes such as parasitic infections or allergic
Results presented as kinetic enzyme-linked immunosorbent assay slope ± SD (duplicate or triplicate determinations).

Table 1. IgE and IgG Antibodies to Extract of Hypoderma lineatum First Instar Larvae

<table>
<thead>
<tr>
<th>Test serum</th>
<th>IgE Response*</th>
<th>% Total counts added (% negative control)</th>
<th>IgG Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radioactive counts bound†</td>
<td>Patient (2/22/96)§</td>
<td>119±5</td>
<td>177±5</td>
</tr>
<tr>
<td></td>
<td>12.62 (765)</td>
<td>1.65 (100)</td>
<td>0.23±0.21</td>
</tr>
<tr>
<td></td>
<td>Patient (6/11/96)§</td>
<td>0.23±0.21</td>
<td>0.23±0.21</td>
</tr>
<tr>
<td></td>
<td>12.75 (773)</td>
<td>48.31</td>
<td>1.65 (100)</td>
</tr>
</tbody>
</table>

*To verify specificity, the patient's serum was added to mixtures of solid-phase and various volumes of fluid-phase extract of H lineatum first instar larvae (HL1); bound IgE was detected with radiolabeled anti-IgE. An extract of natural rubber latex, an unrelated antigen, was used as a fluid-phase negative control for the inhibition experiments. Fluid-phase HL1 extract inhibited the binding of IgE with the solid-phase HL1; in contrast, natural rubber latex extract did not.
†Results are means of duplicates.
‡The control serum was from a man with no history of atopy, no exposure to H lineatum, and low total IgE antibody.
§About 2 months after emergence of the worm.
¶Results presented as kinetic enzyme-linked immunosorbent assay slope ± SD (duplicate or triplicate determinations).
Human control serum from Sigma Chemical Co, St Louis, Mo (No. H1388), clotted male whole blood, nonreactive for human immunodeficiency and hepatitis B and C viruses.

For personal use. Mass reproduce only with permission from Mayo Clinic Proceedings.
ings, pleuritis, pericarditis, and furunculosis. The diagnosis can be confirmed by IgE and IgG responses to *H. lineatum* antigens. Treatment with ivermectin at a dose of 200 μg/kg is beneficial.  

**ACKNOWLEDGMENTS**

We thank Richard T. Jones for performing the IgE antibody immunoassays and immunoblotting experiments, Gail M. Kephart for immunofluorescent staining of the biopsy tissues, Brenda Burkett for valuable assistance in performing the kinetic ELISA, Linda H. Arneson for secretarial assistance, and Cheryl R. Adolphson for editorial assistance.

**REFERENCES**