
Case report

A patient with severe black fly (Simuliidae) hypersensitivity referred for evaluation of suspected immunodeficiency

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Background: Biting flies of the Diptera order and specifically the black fly (Simuliidae family) can be rare causes of severe hypersensitivity reactions.

Objective: To describe a patient referred for evaluation of immunodeficiency whose clinical course is explained by severe Simuliidae hypersensitivity.

Methods: The patient's immune system was investigated using standard laboratory evaluations. Hypersensitivity to Simuliidae was pursued because of historical features of her presentation and was specifically examined by skin prick and intradermal testing with whole body extract.

Results: The patient's history was notable for recurrent and severe seasonal episodes of presumed cellulitis after black fly bites that responded poorly to intravenous antibiotics. One episode was followed by acute inflammatory demyelinating polyneuropathy (Guillain-Barré syndrome) and another by minimal change nephrotic syndrome. The results of immunologic investigations were unremarkable, but cutaneous hypersensitivity to Simuliidae was demonstrated with a 6.5-mm wheel and 35-mm flare reaction to intradermal injection of only 0.0005 μ g of whole body extract.

Conclusion: Similar to Hymenoptera, Simuliidae can rarely result in extreme hypersensitivity and should be considered in appropriate cases. This patient illustrates how severe reactions to insect bites can be misdiagnosed.

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INTRODUCTION

Hypersensitivity to stinging insects of the Hymenoptera order is relatively common, and the incidence of allergic reactions is estimated at 0.4%.¹ Most of these events occur within 4 hours of the sting and involve the skin but can occasionally result in generalized anaphylaxis. Approximately 1% of reactions occur after 4 hours and can include extreme immune-mediated complications, such as serum sickness, nephrotic syndrome,² or acute inflammatory demyelinating polyneuropathy (AIDP; Guillain-Barré syndrome).³ Hypersensitivity reactions to biting, nonstinging insects have also been reported but are markedly less common than to Hymenoptera.^{4,5}

The biting insects of the Diptera order contain several family members, including mosquitoes (Culicidae), houseflies (Muscidae), deer flies (Tabanidae), and black flies (Simuliidae), also known as blood-sucking black flies.⁶ This common name for Simuliidae refers to the painful bite inflicted by the female to obtain a blood meal used to develop her eggs. The insects' saliva contains an anticoagulant, and

the bite is associated with more bleeding than that caused by other flies.⁷ Human bites from Simuliidae are common and are central to transmission of certain vector-borne illnesses, such as onchocerciasis, in certain parts of the world. Simuliidae are found near running water and are extensively distributed in Canada and the northeastern United States, where they proliferate in the springtime.

Although severe allergic reactions to bites from Culicidae, Tabanidae, Muscidae, and Simuliidae are known to occur, they are generally mild.^{4,5} Simuliidae hypersensitivity, however, has been reported in several small series and case reports.^{4,8,9} These individuals experienced mainly local reactions, although a few had flu-like symptoms. In this report, we describe a patient who had extreme hypersensitivity to Simuliidae and was initially referred for evaluation of immunodeficiency because of confusion of her severe reactions with infectious illnesses.

METHODS

In Vitro Assays

Quantitative and functional evaluations of humoral and cellular immunity, as well as complement, were performed by the Children's Hospital Core Laboratory and Clinical Immunology Laboratory using Clinical Laboratory Improvement

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Act-certified assessments. Proliferation of lymphocytes induced by mitogens or antigens was performed as described.¹⁰ Dihydrodamine reduction to evaluate phagocyte function was performed by ARUP Laboratories (Salt Lake City, UT).¹¹ Serologic presence of specific IgE was determined using the ImmunoCap system (Pharmacia, Peapack, NJ) and was performed by the Children's Hospital Core Laboratory or by ARUP Laboratories.

Skin Testing

Whole body extracts of mosquito (Culicidae), deer fly (Tabanidae), housefly (Muscidae), and mayfly (Ephemeroptera) were obtained from Greer Laboratories (Lenoir, NC). Whole body extract of Simuliidae was provided by Dr Thomas Grier and was prepared as a stock solution of 0.1 mg/mL in sterile saline. The black fly source materials used to prepare Greer freeze-dried extract lot XPB40-R1 were whole body insects obtained from an established commercial collector. Insect identity was confirmed by both the collector and a Greer raw material specialist. The insect bodies were milled, defatted with acetone and powdered, then extracted overnight at 2°C to 8°C in bicarbonate buffer, pH 8.0, at a 1:10 (wt/vol) extraction ratio. Soluble products were dialyzed against distilled water and lyophilized. No assessments of allergenic potency were conducted because of a lack of availability of human serum IgE from black fly-sensitive individuals.

Skin prick testing was performed using a Multitest device (Lincoln Diagnostics, Decatur, IL). The patient did not have a wheal-and-flare reaction to skin prick testing with saline but had a 5-mm wheal and 28-mm flare reaction to skin prick testing with 6 mg/mL of histamine (Greer Laboratories). Intradermal testing was performed by injecting 0.05 mL of whole body extracts diluted in isotonic sodium chloride solution using a 27-gauge, 3/8-in needle (Becton Dickinson, Franklin Lakes, NJ).

RESULTS

Case Report

The patient was delivered at 33 weeks' gestation and was small for gestational age. Although she spent her first month of life in a neonatal intensive care unit, she was healthy

during the next 3 years. One year before the start of her medical difficulties, the patient moved to rural Massachusetts from Alaska. In the springtime of her fourth year of life, she was bitten behind her ear by what her parents describe as a black fly and developed erythema and induration within 1 hour of the bite. She was diagnosed as having cellulitis and prescribed antibiotics. Four days later the lesion had not improved, and she developed peripheral weakness and ataxia. She was diagnosed as having AIDP and required endotracheal intubation with mechanical ventilation 2 days later because of ascending weakness and respiratory failure. She recovered from the episode with mild residual neurologic deficit. In the springtime of her fifth year, she once again developed a local reaction after being bitten by what her parents described as black flies, which persisted and was diagnosed as cellulitis. Several days later, she developed facial puffiness and peripheral edema and was diagnosed as having minimal change nephrotic syndrome that responded well to 2 years of corticosteroid therapy. In the springtime of her sixth, seventh, and eighth years, she continued to develop local erythematous, indurated lesions after being bitten by black flies. Individual episodes were characterized by onset of erythema and induration within 1 hour and some elevated body temperature within a few days. She did not experience arthralgias, myalgias, or late-onset generalized rashes. The individual lesions always persisted for more than 1 week, did not display gross evidence of necrosis, and were typically diagnosed as cellulitis and treated with antibiotics. Although all lesions resolved after 3 weeks, it was believed that they responded more rapidly when intravenous antibiotics were used.

After the episode in her eighth year of life, she was suspected of having an immunodeficiency that predisposed her to recurrent cellulitides and was referred for further evaluation. Importantly, the child was typically well in the fall and winter months and never had pneumonia, sinusitis, or deep tissue infections. She has experienced fewer than 5 episodes of otitis media, all of which responded appropriately to oral antibiotics without issue. The chronology of her medical history is summarized in Table 1.

Table 1. Clinical Chronology

Age	Month	Diagnosis	Treatment	Outcome
3 y 1 mo	May	Cellulitis following insect bite	Oral antibiotics	Presented with ataxia 4 days later
3 y 1 mo	May	Acute inflammatory demyelinating polyneuropathy	Intravenous immunoglobulin and mechanical ventilation	Mild neurologic deficit
4 y	April	Cellulitis following insect bite	Oral antibiotics	Presented with worsened swelling days later
4 y	April	Nephrotic syndrome	Prednisolone	Consistent improvement with mild relapses during next 22 mo
5 y 1 mo	May	Cellulitis following insect bite	Oral antibiotics	Complete resolution during 3 wk
6 y	April	Cellulitis following insect bite	Oral antibiotics	Complete resolution during 3 wk
7 y 1 mo	May	Cellulitis following insect bite	Intravenous antibiotics	Complete resolution during 2 wk
8 y 1 mo	May	Cellulitis following insect bite	Intravenous antibiotics	Complete resolution during 2 wk

Immune Studies

Due to the presumed recurrent skin infections, an evaluation of the patient's immune system was undertaken (Table 2). Quantitative immunoglobulin analysis within weeks after a suspected cellulitis episode revealed normal levels of IgG, IgA, and IgM, but an elevated IgE level. Generation of specific antibodies was normal, since antibody directed against her vaccinations (*Haemophilus influenzae* and tetanus) was demonstrated. In addition, she had also demonstrated protective antibody to varicella (IgG, 1.26 IU/mL) 2 years after receiving attenuated varicella vaccination. Cellular immunity was evaluated by lymphocyte subsets and lymphocyte proliferative responses to mitogens (phytohemagglutinin, concanavalin A, and pokeweed mitogen) and antigens (diphtheria and tetanus toxoids), all of which were normal for her age. She also had normal phagocyte oxidative function as determined by dihydroadamine reduction and normal lytic complement activation as determined in a hemolytic assay (CH50). Interestingly, her elevated IgE level decreased markedly with increasing time from the springtime episode.

Allergy Testing

Since all of the patient's episodes occurred only in the springtime and appeared to follow black fly bites, the

diagnosis of black fly hypersensitivity was considered. The patient was tested with extracts of a variety of flies in the Diptera order, including Simuliidae, Culicidae (mosquito), Tabanidae (deer fly), and Muscidae (housefly), as well as a nonbiting, non-Diptera Exopterygota class member Ephemeroptera (Mayfly) used as an insect control. She had negative responses to skin prick testing with all extracts. Intradermal testing was performed, and the patient had a 6.5-mm wheal and 35-mm flare response after injection of 0.0005 μ g of Simuliidae whole body extract (Figure 1). A 6-mm wheal and 12-mm flare were also noted after injection of 0.2 μ g of Culicidae whole body extract. Intradermal injections of the other whole body extracts were negative at 0.2 μ g. Delayed reactivity at any of the percutaneous or intradermal skin test sites, including induration, was not found. In addition, the patient did not have any systemic symptoms of hypersensitivity on the day of testing or in the week following testing. Intradermal injection of 0.0005 μ g of Simuliidae extract into 3 otherwise healthy control individuals, 2 of whom had experienced numerous black fly bites without significant reaction, was negative. Furthermore, intradermal injection of control donors with as much as 0.5 μ g of Simuliidae extract failed to elicit any wheal or flare response. The patient's serum was also evaluated for the pres-

Table 2. Immunodeficiency Evaluation

	Months after episode*				Reference range	
	0	3	7	9	5th percentile	95th percentile
Total leukocytes, cells/ μ L	6,840	6,910			4,800	10,500
Neutrophils, cells/ μ L	2,941	3,386			1,776	8,085
Lymphocytes, cells/ μ L	2,804	2,488			624	5,040
CD3+, %		68			65	75
CD3+/CD4+, %		32			35	50
CD3+/CD8+, %		35			18	30
CD3-/CD16+/CD56+, %		14			5	15
CD19+, %		15			5	10
Monocytes, cells/ μ L	410	276			192	840
Eosinophils, cells/ μ L	342	345			0	525
IgG, mg/dL	805	717		741	639	1,344
IgG1		494			456	938
IgG2		150			163	513
IgG3		73			26	113
IgG4		14			1	95
IgA, mg/dL	161	123			70	312
IgM, mg/dL	136	127			40	240
IgE, IU/mL	6,820	1,860	733	227	0	200
Serotypes in heptavalent vaccine		3/6 tested				
Serotypes not in heptavalent vaccine		4/9 tested				
Anti-Hib polyribosylphosphate, ng/mL		7,862			>1,000	>1,000
Antitetanus titer, IU/mL		0.12			0.15	7.00
Unstimulated DHR reduction, %	0					
Stimulated DHR reduction, %	98				>90	>90

Abbreviations: DHR, dihydroadamine; Hib, *Haemophilus influenzae* type b.

* Evaluations were performed following the most recent episode, which occurred during her eighth year of life.



Figure 1. Intradermal skin test results. Wheal-and-flare response 20 minutes after intradermal injection of 0.0005 μ g of Simuliidae extract (position 1) into the dermis over the right deltoid. Other injections shown were 0.2 μ g of Muscidae (position 2), Ephemeroptera (position 3), Tabanidae (position 4), and Culicidae (position 5) extracts and isotonic sodium chloride solution (position 6).

ence of specific IgE recognizing various Hymenoptera (*Apis mellifera*, *Bobmus* spp, *Vespa* spp, *Dolichorespula arena-ria*, and *Dolichorespula maculata*) and was found to be negative (<0.35 kU/mL).

DISCUSSION

In this report, we describe a patient who experienced extensive cutaneous reactions each spring after presumably being bitten by black flies. After 2 of these episodes, she also developed more severe sequelae, including AIDP and nephrotic syndrome. The severity and frequency of the cutaneous reactions, which were believed to be complicated by bacterial infection, led to a referral for consideration of immunodeficiency. Her immunologic evaluation performed soon after an episode was not significant for defective immunity but revealed an elevated IgE level (Table 2). Importantly, her total IgE level decreased as time passed relative to her last identified black fly bite, suggesting a role for IgE in disease pathogenesis. These features led us to consider biting insect hypersensitivity.

The patient's family provided a description of the particular fly in question that was consistent with Simuliidae. Furthermore, the occurrence of reactions in April and May combined with the fact that the family resided in a rural region near running water specifically prompted evaluation of Simuliidae hypersensitivity. Despite this, the patient was evaluated with a variety of biting fly whole body extracts, but

she only reacted to Simuliidae at a significantly dilute concentration. Importantly, her episodes were most probably not caused by Hymenoptera, because she does not have a history of Hymenoptera stings and tested negative with IgE against Hymenoptera venom. Similarly, the episodes are presumably not due to penicillin hypersensitivity, because she does not have serum IgE directed against penicillin and has successfully tolerated penicillins in recent years for otitis media. Although studies of mosquito whole body compared with salivary gland extracts suggest that pure biting insect testing reagents are needed for accurate diagnosis,¹² our patient's compelling history, isolated skin test reactivity, and negative responses in control subjects suggest Simuliidae bites are central to her disease pathogenesis. Thus, it is most probable that the patient's episodes represent hypersensitivity to antigenic components of Simuliidae. This case highlights the importance of considering Simuliidae hypersensitivity given the appropriate history.

It is unclear, however, why the patient experienced her most severe hypersensitivities (AIDP and nephrotic syndrome) during her first 2 black fly seasons in Massachusetts (Table 1). We speculate that lack of subsequent severe reactions may be related to the prolonged course of systemic corticosteroids she received for treatment of nephrotic syndrome. In support of this theory, her reactions at the ages of 7 and 8 years were more severe than those at the ages of 5 and 6 years, when she was receiving systemic therapy for her renal disease. The fact that there have been no subsequent flares of her nephrotic syndrome, even after bites, suggests that there may have been some modification of her underlying immunologic process. Alternatively, the dose of antigen she has been exposed to in recent years may have been less relative to her total body weight and has thus not exceeded a specific threshold that was surpassed when she was younger and weighed less.

During the past 35 years, there have only been limited reports of Simuliidae hypersensitivity.^{4,8,9,13} Several patients with suspected black fly allergy have been found to have wheal-and-flare reactions on skin testing with Simuliidae whole body extract. Most of these individuals had severe local reactions, with a subset experiencing systemic effects such as lymphadenitis, fatigue, and fever. A single patient was presumed to have Simuliidae-induced anaphylaxis, but the presentation was confused by the presence of Hymenoptera-specific IgE.⁴ We are unaware of any previous examples of extreme delayed hypersensitivity as described in this report. In this regard, we are also unaware of any descriptions of either AIDP or nephrotic syndrome following bites of Diptera family members.

In contrast to our patient's unusual severe delayed reactions, several reports have documented severe immediate hypersensitivity to other Diptera family members.¹⁴⁻¹⁸ Although both immediate and delayed hypersensitivity reactions to biting insects may be underestimated, the diagnosis is too rare to determine if the ratio of immediate to delayed reactions caused by Diptera is similar to that resulting from

Hymenoptera stings. Despite the paucity of cases, the uniqueness of this report suggests that the ratio of these reactions may be a constant across insect families.

The fact that our patient reacted to Culicidae at relatively high concentration is not necessarily a surprise. Simuliidae is a member of the suborder Nematocera of which Culicidae is also a member. All the other Diptera members evaluated in this patient are members of the suborder Brachycera, which are more distantly antigenic and failed to induce positive skin test responses in the patient. Thus, we speculate that the specific antigen present in Simuliidae is probably common to the entire subfamily as a similar structural entity but shows notable diversity across the broader order of Diptera. Recent advances in the understanding of Nematocera hypersensitivity provide avenues to explore these hypotheses.¹⁹

The patient described in this case probably received the diagnosis of cellulitis in error and was unnecessarily treated with intravenous antibiotics. She also was inappropriately presumed to have an immune deficiency and thus referred for an extensive evaluation. Although Simuliidae reactions may be rare, severe local reactions to stinging insects and other biting insects are not,^{20,21} and appropriate diagnosis and management can help prevent morbidity, confusion, and inappropriate therapies. Patients with large local reactions to bites and stings are best managed with specific avoidance strategies and postexposure corticosteroids.^{20,21}

The patient described in this report is unique in the type and severity of the hypersensitivity she repeatedly experienced in response to Simuliidae bites. The case demonstrates the confusion in diagnosing this condition and suggests that extreme hypersensitivity to Diptera members is a legitimate consideration.

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