

soundingly refutes that misconception. In such areas, unless a spider is caught in the act of biting and is subsequently identified as capable of producing an ulcer, ulcers should be attributed to other causes, many of which we listed in our article.

On the other hand, as compared with Connecticut, Missouri is a loxosceles hotbed. We appreciate and acknowledge Dr. Wasserman's comments about the occasional severe reaction in persons who receive a loxosceles bite. Fortunately, such reactions are rare, but unfortunately, there is no proven effective treatment for them. Our point is that even in areas where the spider is endemic, physicians need to keep an open mind and consider more likely diagnoses when faced with possible

but undocumented necrotic arachnidism. Doing so helps to prevent diagnostic mistakes, such as a missed diagnosis of necrotizing fasciitis.<sup>3</sup>

David L. Swanson, M.D.

Mayo Clinic  
Scottsdale, AZ 85259

Richard S. Vetter, M.S.

University of California  
Riverside, CA 92521

1. Kaston BJ. Spiders of Connecticut: state geological and natural history survey of Connecticut. Bulletin 70. Hartford, Conn.: Department of Environmental Protection, 1981:1-1020.

2. Anderson PC. Loxoscelism threatening pregnancy: five cases. Am J Obstet Gynecol 1991;165:1454-6.

3. Coroner: infection, not spider bite, caused man's death. Associated Press, June 30, 2004.

## Antibody Response to Aerosolized Transgenic Human Alpha<sub>1</sub>-Antitrypsin

**TO THE EDITOR:** The Department of Health and Human Services has recommended the development of recombinant counterparts to blood-derived therapeutic human proteins.<sup>1</sup> In response to this recommendation, sheep-derived transgenic human alpha<sub>1</sub>-antitrypsin (AAT) was developed for aerosolized delivery to the lungs. A flock of AAT-transgenic sheep was established by means of the nuclear transfer into sheep ova of normal human

AAT complementary DNA linked to the sheep  $\beta$ -lactoglobulin promoter.<sup>2</sup> Human AAT was isolated from whey from the milk of transgenic sheep and purified (99.9 percent) by sequential chromatography. The major impurities in the final product were sheep AAT and sheep alpha<sub>1</sub>-antichymotrypsin (ACT), in concentrations ranging from 6.7 to 18.7 mg per liter and 60.3 to 75.8 parts per million, respectively. Purified transgenic AAT differs from normal human AAT only in the structure of three N-linked carbohydrates.

People with a deficiency of AAT are at risk for emphysema as a result of low levels of AAT in the lungs in the presence of neutrophil elastase.<sup>3</sup> To evaluate the safety and immunogenicity of transgenic human AAT as augmentation therapy, in two sequential studies we administered 250 mg of aerosolized transgenic AAT daily for eight weeks to 41 subjects with AAT deficiency. A systemic antibody response was defined as a serum IgG antibody titer that exceeded the titer in the sample obtained at the baseline screening by a factor of 4 or more. Among the 41 subjects, antibody responses to sheep AAT occurred in 10, and antibody responses to sheep ACT occurred in 32 (Table 1). No subject had antibody to transgenic human AAT. Four subjects dropped out of the second study because of dyspnea and a decline in lung function. Antibody responses to both major impurities developed in three of these four subjects, including one in whom hypoxemia

**Table 1. Adverse Events and Systemic Antibody Responses among a Total of 41 Subjects.**

Event or Response	No. of Subjects (%)
≥1 Event	31 (76)
Most frequent adverse events	
Cough	11 (27)
Dyspnea	11 (27)
Drug-related adverse event	8 (20)
Dyspnea	4 (10)
Increased cough	3 (7)
Discontinuation because of drug-related adverse event	4 (10)
Systemic antibody response	
Positive response to sheep AAT*	10 (24)
Positive response to sheep ACT*	32 (78)

\* A positive response was defined as an increase by a factor of 4 or more in serum titer between the sample obtained at baseline screening and that obtained after treatment with the study drug.

developed and pulmonary infiltrates were detected on computed tomography of the chest. One subject who participated in both studies had an antibody response to sheep AAT during the second study, with the highest rise in titer (by a factor of 12) of all subjects and a rise in the titer of antibodies to ACT by a factor of more than 16. This subject withdrew from the second study within 10 days after receiving the study drug because of exertional dyspnea and hypoxemia, both of which resolved 4 days after the study drug was discontinued.

We observed systemic antibody responses to nonhuman protein that was present in very low concentrations in a sheep-derived transgenic human AAT formulation. Among subjects who withdrew from the study, there was a possible relationship between drug-related adverse events and a high-titer antibody response. In the absence of a control group receiving placebo, this relationship is uncertain. The clinical symptoms and secondary antibody responses that occurred in the one subject who participated in both studies suggest that reexposure could result in intolerance of nonhuman-protein impurities. These observations may represent an important obstacle to the development of transgenic human proteins for therapeutic use. Strategies to evaluate immune-mediated toxic effects of preparations of transgenic protein should

be established to ensure the development of safe and effective therapies.

L. Terry Spencer, M.D.

University of Florida College of Medicine  
Gainesville, FL 32610-0296

John E. Humphries, M.D.

Bayer HealthCare  
Research Triangle Park, NC 27709

Mark L. Brantly, M.D.

University of Florida College of Medicine  
Gainesville, FL 32610-0296

for the Transgenic Human Alpha<sub>1</sub>-Antitrypsin Study Group

Dr. Spencer reports having served as a scientific advisor for Aventis Behring and Bayer HealthCare (which produces plasma-derived alpha<sub>1</sub>-antitrypsin and has a contractual relationship with PPL Therapeutics, the manufacturer of the transgenic alpha<sub>1</sub>-antitrypsin used in the studies in this report). Dr. Brantly reports having served as a scientific advisor for Aventis Behring, Bayer HealthCare, PPL Therapeutics, the American Red Cross, and Baxter Biologics.

1. Nightingale SD. Summary of the Advisory Committee on Blood Safety and Availability Meeting on April 27 and 28, 1998. Washington, D.C.: Department of Health and Human Services, 1998. (Accessed April 21, 2005, at <http://www.hhs.gov/bloodsafety/summaries/sumapr98.html>.)

2. Wright G, Carver A, Cottom D, et al. High level expression of active human alpha-1-antitrypsin in the milk of transgenic sheep. *Biotechnology (N.Y.)* 1991;9:830-4.

3. Blank CA, Brantly M. Clinical features and molecular characteristics of alpha 1-antitrypsin deficiency. *Ann Allergy* 1994;72:105-20. [Erratum, *Ann Allergy* 1994;72:305.]

Correspondence Copyright © 2005 Massachusetts Medical Society.

#### INSTRUCTIONS FOR LETTERS TO THE EDITOR

Letters to the Editor are considered for publication, subject to editing and abridgment, provided they do not contain material that has been submitted or published elsewhere. Please note the following: •Letters in reference to a *Journal* article must not exceed 175 words (excluding references) and must be received within three weeks after publication of the article. Letters not related to a *Journal* article must not exceed 400 words. All letters must be submitted over the Internet at <http://authors.nejm.org>. •A letter can have no more than five references and one figure or table. •A letter can be signed by no more than three authors. •Financial associations or other possible conflicts of interest must be disclosed. (Such disclosures will be published with the letters. For authors of *Journal* articles who are responding to letters, this information appears in the original articles.) •Include your full mailing address, telephone number, fax number, and e-mail address with your letter.

Our Web address: <http://authors.nejm.org>

We cannot acknowledge receipt of your letter, but we will notify you when we have made a decision about publication. Letters that do not adhere to these instructions will not be considered. Rejected letters and figures will not be returned. We are unable to provide prepublication proofs. Submission of a letter constitutes permission for the Massachusetts Medical Society, its licensees, and its assignees to use it in the *Journal's* various print and electronic publications and in collections, revisions, and any other form or medium.