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CASE REPORT

PATHOLOGY/BIOLOGY

Klára Törő,[1] *M.D., Ph.D.; Katalin Borka,*[2] *M.D., Ph.D.; Magdolna Kardos,*[2] *M.D.; István Kristóf,*[1] *M.D.; and Péter Sótonyi,*[1] *M.D., D.Sc.*

Expression and Function of C5a Receptor in a Fatal Anaphylaxis After Honey Bee Sting*

ABSTRACT: The mechanisms leading to death from anaphylaxis after insect sting involve antigen cross-linkage of antibody molecules that activate immunoglobulin receptors on inflammatory cells. The aim of our study was to investigate the pathomorphology and the expression of C5aR in fatal anaphylaxis in a patient after a fatal insect sting. A 38-year-old women was stung by a honeybee. C5R1 expression was detected in many dilated capillaries in the lungs. Pulmonary epithelial cells did not bind the monoclonal antibody for C5R1; however, intensive cytoplasmic staining was detected in endothelial cells. The findings of this case provide evidence for an active role of complement in fatal anaphylaxis elicited by bee venom. C5aR detection could be useful in the identification of sudden death cases because of unwitnessed fatal insect sting cases. Authors can recommend this immunohistochemical analysis on all sudden unexpected deaths outdoors where a possible bee sting might occur.

KEYWORDS: forensic science, complement C5a, C5aR, fatal anaphylaxis, honey bee sting, medico-legal autopsy

Anaphylactic responses to insect stings is an acute systemic allergic reaction occurring as a result of the release of chemical mediators after an immunologic reaction, typically IgE mediated (1). IgE antibody production mediates most human anaphylaxis by increasing target cell responsiveness to vasoactive mediators; however, human anaphylaxis has been described in which there was no evidence of antigen-specific IgE antibodies or mast cell degranulation products, such as histamine and tryptase.

Stings of *Hymenoptera* commonly cause anaphylaxis (2,3). In stinging bees, this venom delivery system is a powerful defense against vertebrates, which in the case of humans can be life threatening when the individual is sensitized to allergens in the venom. Glycoproteins from the honeybee (*Apis mellifera*), such as phospholipase A_2 and hyaluronidase, are well-known bee venom allergens (4,5).

Human complement is a complex network of soluble and membrane-associated serum proteins that participate in highly regulated humoral and cellular immune responses to infectious organisms, tissue damage by chemical, physical, or radiation exposure, and substances not recognized as "self." The complement activation pathway produces C5a complement fragments, a 74 residue pro-inflammatory polypeptide produced during activation of the complement cascade of serum proteins in response to foreign substances (6,7). C5a was first described as a classical anaphylatoxin capable of stimulating the secretion of histamine from mast cells (8), and it was later identified as a potent neutrophil (9,10) and macrophage (11) chemoattractant. Now, C5a is recognized as a pleiotropic molecule that can modulate the activity of many cell types. It has a broad range of biological functions both inside and outside of the immune system. The role of anaphylatoxin C5a receptors in anaphylaxis is suggested by findings from different animal models (12,13).

The aim of our study was to investigate the pathomorphology and the expression of C5aR in fatal anaphylaxis after insect sting.

Case Report

History

A 38-year-old women was stung by a honeybee. The eyewitnesses reported dizziness, respiratory failure, and death within 4 min after the sting. There was no history of any previous reactions to past stings and no well-characterized underlying medical conditions. The victim has no other allergic diseases, asthma, or skin reactions.

Autopsy and Histology

The autopsy was performed 48 h after death; however, the body was stored at +4°C immediately after death. Brain, heart, liver, kidney, and intestines did not show any signs of pathomorphological changes. There was no evidence of emphysema or pulmonary edema, or significant mucous bronchial secretion. Pathomorphological changes of cardiac diseases were not detected. Blocks of lung, pharynx, epiglottis, brain, heart, liver, kidney, and intestines were collected during the postmortem investigation. Tissue specimens were fixed in 4% formaldehyde and paraffin embedded. Sections (5 μm) were cut and screened with hematoxylin–eosin and Giemsa staining. Microscopically, laryngeal and tracheal subepithelial capillaries showed minimal dilatation and interstitial edema without any signs of inflammation (Fig. 1*a*); however, it was not seen

[1]Department of Forensic and Insurance Medicine, Semmelweis University, 1091-Hungary, Budapest Üllői út 93, Hungary.
[2]2nd Department of Pathology, Semmelweis University, 1091-Hungary, Budapest, Üllői út 93, Hungary.
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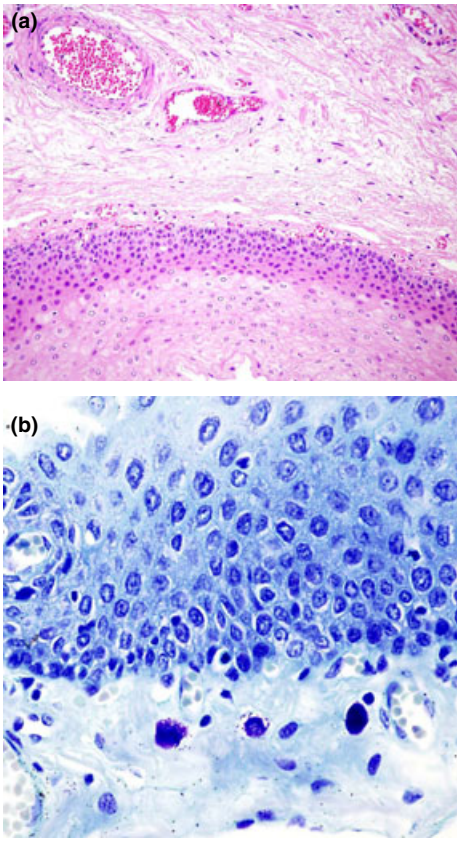

FIG. 1—*(a) Dilated capillaries in laryngeal mucosa with hematoxylin–eosin staining (×200). (b) Degranulated mast cells in the subepithelial connective tissue in the larynx with Giemsa staining (×600).*

macroscopically based on the minimal tissue changes. Many mast cells were seen in the airways with Giemsa staining (Fig. 1*b*).

Immunohistochemistry

The sections were deparaffinized for immunoperoxidase staining with xylene and 100% ethanol. Endogenous peroxide was blocked with 0.3% hydrogen peroxide in methanol for 10 min at room temperature. The slides were treated for 30 min with target retrieval solution (DAKO, Glostrup, Denmark) in a microwave oven. The following steps are carried out in a Ventana ES automatic immunostainer (Ventana Medical Systems, Inc., Tucson, AZ) using the reagents provided by the manufacture. The primary antibody was a mouse monoclonal C5R1 (dilution: 1:100, 32 min at 42°C; Abcam, Cambridge, UK). Binding of the secondary antibody was detected by the substrate (DAB). The slides were counterstained with hematoxylin, rinsed in distilled water, dehydrated through 95% ethanol for 2 min, 100% ethanol for twice for 3 min, and cleared in xylene twice for 5 min. Coverslips were fixed with mounting medium. Positive controls recommend by the manufacturer (Abcam) were used to confirm correct immunohistochemical staining for C5R1. These included samples of liver, intestinal tract, lung, and spleen. For negative control, the primary antibody was omitted, and either the antibody diluent alone or isotype-matched IgG serum was used.

C5R1 expression was detected in many dilated capillaries in the lungs (Fig. 2). Pulmonary epithelial cells did not bind the monoclonal antibody for C5R1; however, intensive cytoplasmic staining was detected in endothelial cells (Fig. 3). No positive reactions were identified in tissues from the liver, heart, or kidney. Control tissues obtained from a suicide case were compared, and no antibody to C5R1 was detected in the epithelial cells or capillaries.

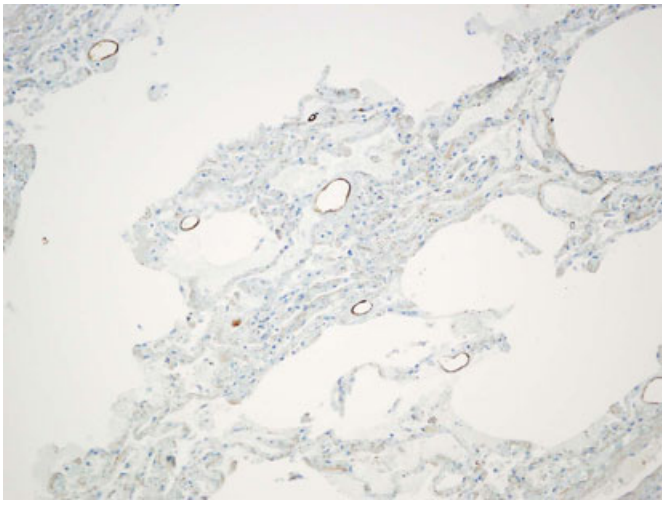

FIG. 2—*C5R1 expression was detected in many dilated capillaries in the lungs (×200).*

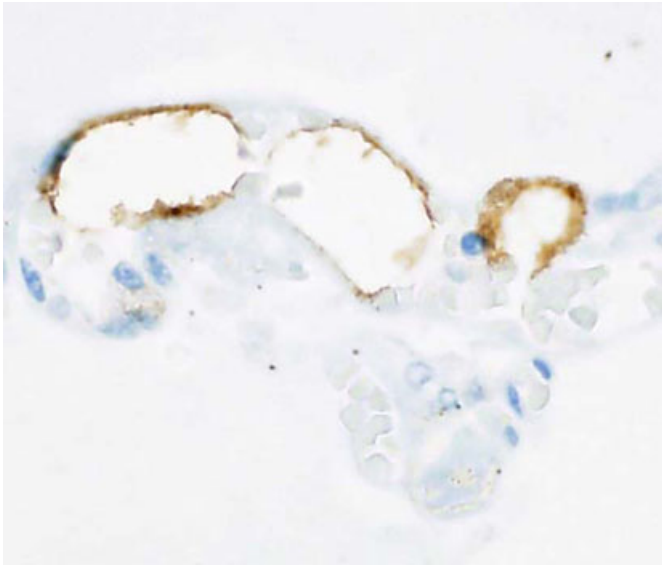

FIG. 3—*Cytoplasmic positivity was detected in endothelial cells (×600).*

Discussion

The findings of our case report suggest a role for complement C5a in fatal human anaphylaxis. Our postmortem results suggest that the anaphylactic responses were primarily in the blood vessels. These findings support the mechanism of rapid induction of physiological responses with significant mast cell degranulation leading to death.

This case is the first report to our knowledge in which C5a was detected postmortem in fatal human anaphylaxis after an insect sting; however, there are studies (2,5) dealing with the clinical diagnosis of anaphylaxis include serial measurements of total tryptase in serum during an anaphylactic episode and measurement of baseline total tryptase levels after the episode. Hymenoptera venom was found to be the second most frequent cause of anaphylaxis

(29%), and most of the patients with hymenoptera venom had no history of autopsy (5).

During medico-legal autopsy, anaphylaxis induced by insect sting needs careful investigation. Because the fatal process can be very rapid, it can happen in the absence of witnesses and the pathomorphology is very poor with no characteristic features. Results presented here could be useful for examining unwitnessed deaths in which there is evidence of an insect sting to differentiate the death in response to stings from other sudden death causes.

Other circumstances can elicit anaphylaxis: intravenous injection of some liposomal drugs; and diagnostic agents. Micelles and other lipid-based nanoparticles can cause acute hypersensitivity reactions (HSRs) in a high percentage (up to 45%) of patients resulting in hemodynamic, respiratory, and cutaneous manifestations (14). The phenomenon can be explained by activation of the complement system on the surface of lipid particles, leading to anaphylatoxin (C5a and C3a) liberation and subsequent reactions of mast cells, basophils, and possibly other inflammatory cells in blood.

In conclusion, the findings of this case provide evidence for an active role of complement in fatal anaphylaxis elicited by bee venom. Examination of the airway and parenchymal vessels for C5a expression might aid in diagnosis of rapid death because of insect sting. C5aR detection could be useful in the identification of sudden death cases because of unwitnessed fatal insect sting cases. We can recommend this immunohistochemical analysis on all sudden unexpected deaths outdoors where a possible bee sting might occur, and this kind of cause of death could not be seen at medico-legal autopsy except microscopically or by special immuno stains.

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Additional information and reprint requests:
Klára Törő, M.D., Ph.D.
Department of Forensic and Insurance Medicine
Semmelweis University
Budapest Üllői út 93
1091-Hungary
Hungary
E-mail: torok@igaz.sote.hu