Editorial

Can we improve laboratory testing for anaphylaxis so it can have an immediate pragmatic benefit?

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Abbreviations: ECF-A, eosinophil chemotactic factor of anaphylaxis; PAF, platelet activating factor platelet activating factor; SRS-A, slow-reacting substance of anaphylaxis; IgE, immunoglobulin E; PGD_2, Prostaglandin D_2

Introduction

When a specific allergen gains access to the circulation, the allergen can react with basophils of the blood and mast cells in the tissues located immediately outside the small blood vessels, if the basophils and masts cells have been sensitized by attachment of IgE reagins. This can be followed by a widespread allergic reaction throughout the vascular system and closely associated tissues, which is referred to as anaphylaxis. Presently, the diagnosis of anaphylaxis is based on the patients clinical presentation. Laboratory studies are rarely of immediate help. However, there are circumstances in which the diagnosis of anaphylaxis is unclear, or the medical personnel arriving at the patient's side, EMT or Paramedic, may not have the educational level to recognize the clinical presentation of the patient: swollen tongue, inability to speak with stridor, may have another causation than anaphylaxis. Such lack of understanding can lead to a line of treatment, such as giving epinephrine for a misdiagnosis of anaphylaxis, in a patient who has a bleeding AV malformation of the brain with unfortunate consequences. If we could develop a laboratory test that could be used by medical personnel, such as EMTs or Paramedics, who first arrive at the scene and who are unsure of the diagnosis of anaphylaxis, this could prove to be most beneficial to the quality of care afforded the patient. Such a laboratory test would focus on some of the mediators of immediate hypersensitivity reactions, such as histamine, tryptase, the cysteinyl leukotrienes, and prostaglandin D_2.

Pathogenesis

Anaphylaxis is a type 1 hypersensitivity reaction, which is also known as immediate Hypersensitivity, because the reaction is immediate. It is a sensitization reaction characterized by signs and symptoms of an allergic reaction that typically occurs 15 to 30minutes after exposure to an allergen (antigen). Mast cells and basophils are the principle effector cells, although other cell types, including eosinophils, neutrophils, macrophages, epithelial cells, endothelial cells and lymphocytes are involved in promoting and sustaining allergic inflammation. Mast cells and basophils were once thought to be closely related cell types, however, recent evidence suggest mast cells are derived from mononuclear cell precursors and bear receptors for stem cell factor, whereas basophils develop in the bone marrow. Basophils enter the circulation as mature cells, whereas mast cells undergo maturation in the tissues where they reside as mature cells. Mast cells are found throughout the body in loose connective tissue. There are two phenotypically distinct populations of mast cells, called MCt and MCtc, which are based on their content of tryptase (MCt) and tryptase and chymase (MCtc), respectively. MCt cells are found at mucosal surfaces at the sites of allergic inflammation, often adjacent to T lymphocytes, whereas MCtc cells are more numerous in sub mucosal sites and in connective tissues and are not associated with allergic inflammation or T lymphocytic infiltration.

The mast cells are filled with vesicles or granules containing potent vasoactive, Pro inflammatory chemical mediators, especially histamine that produce inflammation when they are released. Mast cells typically contain up to 10 times more histamine per cell than basophils. The release of the contents of the proinflammatory chemical mediators from the mast cells and basophils is related to IgE receptors, which cover the surface of these cells. Mast cells may have between 40,000 to 500,000 IgE receptors on their surface. The basophils contain granules, which contain acid muco polysaccharide (heparin), histamine, and peroxidase. Basophils synthesize and store histamine and eosinophil chemotactic factor of anaphylaxis (ECF-A). In addition, at the time of exposure to a sensitizing allergen, basophils synthesize and release slow-reacting substance of anaphylaxis (SRS-A) and probably platelet activating factor (PAF), however, they do not store them. ECF-A leads to the accumulation of eosinophils, which contain substances that tend to counteract these mediators. Basophils are also involved in some delayed hypersensitivity reactions, 'cutaneous basophil hypersensitivity,' such as contact allergies, in which they appear to undergo a different type of degranulation response. The principle antibody which mediates the anaphylaxis (hypersensitivity) reaction is immunoglobulin E (IgE). IgE is produced by specialized plasma B cells. Typically, IgE circulates in the blood in very small amounts. When an individual is exposed to an allergen, selected plasma B cells produce allergen specific IgE. IgE binds readily to basophil and mast cell membranes.

When a specific antigen reacts with the membrane-bound IgE, degranulation occurs with the release of mediators of immediate hypersensitivity (e.g., histamine, SRS-A, PAE, heparin, and ECR-A). The initial incident during an anaphylactic reaction is the cross-linking of two IgE receptors to one antigen on the mast cells and basophils located at the site of the allergen's entry into the body. The primary mechanism of release of preformed mediators from the mast cells and basophils involves transmembrane signaling, mediated by the cell surface IgE antibodies bound to the high-affinity receptors for IgE. The cross-linking of IgE and the antigen leads to trans membrane signaling, which causes an increase in intracellular calcium (Ca^2) concentration that results in immediate, massive, local mast cell degranulation of preformed and newly formed proinfammatory mediators. Basophils undergo the same degranulation process with the release of histamine, SRS-A, PAE, heparin and ECF-A. The principle

preformed mediators released from the mast cells are histamine, tryptase, and chymase (neutral proteases),acid hydrolases (e.g. hexosaminidase), heparin, and chondroitin sulfates (proteoglycans), and numerous cytokines and chemo attractants including IL-3, IL-4, TNF-α, and IL-8. The exposure of the mast cells to the allergen also leads to the manufacture and subsequent release of mediators including superoxide, thromboxanes, bradykinin, cyclooxygenase (prostaglandin), lipoxygenase (leukotriene) products of arachidonic acid metabolism and interleukins. One of the most important mediators of type I hypersensitivity is histamine. Histamine binds to H_1 (histamine I), H_2, H_3, and H_4 receptors, which are located on many types of cells. Mast cells have receptors for H_1, H_2, and H_3, with H_1 being the most active.

Basophils primarily express H_2 receptors, whereas neutrophils, eosinophils, monocytes and macrophages have both H_1 and H_2 receptors. Histamine binding to H_1 receptors triggers increased vascular permeability, vasodilatation (flushing), urticaria formation (hives), smooth muscle constriction (bronchconstriction), increased mucus secretion And pruritus (itching), and increased gut permeability. Histamine binding to H_2 receptors produces effects opposite to H_1 receptors in some tissues causing smooth muscle relaxation in the lower airways, augments gastric acid secretion from parietal cells, and in high concentrations has an inhibitory effect on inflammatory cells, decreasing granulation and decreasing neutrophil chemotaxis. H_3 receptors are located in the brain, spinal cord and on sensory neurons such as postganglionic cholinergic nerves in lung bronchi. H_4 receptors are found on immune system cells, such as dendritic cells, eosinophils, T cells, monocytes, macrophages and natural killer cells, as well as hematopoietic cells, such as the spleen, thymus, bone marrow, and blood leukocytes. H_4 receptors are very attracted to histamine and are also involved in chemotaxis and inflammatory response.

Clinical presentation of type I hypersensitivity

Hypersensitivity reactions are grouped into four types according to the effector mechanisms by which they are produced. The effectors for types I, II and III hypersensitivity reactions are antibody molecules, whereas type IV reactions are mediated by antigen specific effector T cells. In this editorial we will address only type I hypersensitivity responses. In a sensitized host, an individual with IgE responses to antigens, re-exposure to antigen leads to type I hypersensitivity responses only in the mast cells and basophils exposed to the antigen. If the allergen is inhaled the resulting type I hypersensitivity response will consist of bronchconstriction and increased mucus secretion (asthma and allergic rhinitis); ingestion of the antigen will cause increased peristalsis and secretion (diarrhea and vomiting); and the presence of subcutaneous antigens initiates vascular permeability and swelling (urticaria and angioedema). Blood borne antigens cause systemic mast cell and basophil cell activation, which leads to increased vascular permeability, hypotension, tissue swelling, and smooth muscle contraction, which are characteristics of systemic anaphylaxis.

Anaphylaxis clinically can be diagnosed in a patient who has an acute onset of cutaneous signs of immediate hypersensitivity, along with hypotension or respiratory compromise in the apparent absence of allergen exposure; the rapid onset of hypersensitivity signs involving at least two organs from among the cutaneous, gastrointestinal, respiratory, and cardiovascular systems after exposure to a likely allergen; or the rapid onset of hypotension after exposure to a known allergen. In many people, the type I hypersensitivity reactions are minor, manifesting as hives (urticaria), seasonal allergic rhinitis, eczema or mild bronchconstriction. However, others will experience far more serious manifestations, such as tightening of the throat due to laryngeal edema (stridor), localized edema, wheezing and tachycardia, such as is associated with severe airway reactions. In a few highly allergic people, the type hypersensitivity reaction can be expressed as a life threatening allergic reaction known as anaphylaxis. These latter reactions are commonly associated with Hymenopter(stinging) insects; Polistes wasps; honeybees; fire ants; hornets and yellow jackets; serum proteins, including γ-globulin, insulin, vaccines, enzymes, such as penicillinase, local anesthetics; ingestion of seafood, especially shellfish; nuts, such as peanut allergic reactions; eggs; fruit, especially citrus and strawberries; nickel; aluminum; zinc; and medications, such as penicillin, penicillin analogues, radiographic contrast media, aspirin, indomethacin, and other analgesics and allergenic extracts.

Laboratory diagnosis

From a laboratory perspective there are two assessments which have pragmatic value, histamine and tryptase levels. Plasma histamine levels increase within 10minutes of onset of symptoms, however, because histamine is rapidly metabolized, its levels fall within 30minutes. Urinary histamine levels are generally not dependable, as the test can be affected by diet and by bacteria in the urine. It also would have little application for first responders at a scene. Urinary histamine metabolites measurement is a better test but is generally not available and would have little value for first responders. An increased level of total tryptase in serum, which peaks at 15 to 60minutes after the onset of anaphylaxis and then declines, with a half-life of about 2hours, remaining elevated for up to 12hours, indicates mast cell activation has occurred. The severity of the mast cell activation is the primary determinant of the clinical severity for the patient. Although, an increased serum total tryptase level during systemic anaphylaxis may be useful for distinguishing anaphylaxis from other conditions in the differential diagnosis, elevations do not occur in some cases of anaphylaxis, especially after food ingestion. It also must be remembered, detecting the rise of histamine or tryptase levels can be difficult, and some patients might have a rise in one but not the other. In one emergency department study evaluating patients with acute allergic reactions, 42 of 97 had elevated histamine while 20 had elevated tryptase levels. There was also no correlation shown between the levels of tryptase and histamine. There are other biomarkers of type I hypersensitivity reactions being studied. These include platelet activating factor (PAF), bradykinin, chymase, mast cell carboxypeptidase A3, dipeptidyl peptidase I, IL-33 and other cytokines, leukotrienes and prostaglandins. Low levels of the PAF acetylhydrolase have been reported in fatal anaphylaxis, and failure of this enzyme to inactivate PAF may help identify individuals at risk for severe or even fatal anaphylaxis. Another consideration for laboratory analysis is Prostaglandin D_2 (PGD_2). PGD_2 is the principal COX-catalyzed product of arachidonic acid secreted by activated mast cells, but it is not made by basophils. It is rapidly metabolized with its urinary metabolites being elevated during anaphylaxis.

Treatment

Although, the purpose of this editorial is to address the clinical, pathophysiology, and laboratory presentation of type I hypersensitivity

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reaction, it is essential we have a very brief discussion of its treatment, for it has an impact on the essence of insufficient laboratory support to distinguish anaphylaxis from a variety of disorders whose clinical presentation overlaps with a type I hypersensitivity reaction. Treatment for prehospital patients with symptoms of severe anaphylaxis should first receive standard interventions, such as high-flow oxygen, cardiac monitoring and intravenous access. Depending on the severity of the clinical presentation, there are a variety of pharmacologic agents, which can be used, such as antihistamines, β-adrenergic, corticosteroids, anticholinergics, and anti-immunoglobulin E therapy (IgE blocker therapy). Antihistamines, such as diphenhydramine (Benadryl) are used to block the effect of histamine. This action decreases vascular permeability and bronchconstriction. β-Adrenergic sympathomimetics are used to decrease bronchconstriction and bronchospasm. Epinephrine is an adrenergic agent (α, β_1 and β_2) given subcutaneously or intravenously during an acute allergic reactions, especially after food or bee sting reactions. Corticosteroids are used to decrease the inflammatory response. Anticholinergics are used to block the parasympathetic system and thus allow greater sympathetic activity. Such blockage of parasympathetic activity indirectly causes bronchodilation. Anti-immunologolbulin E therapy is used in patients who have severe persistent asthma, which is not the subject of this editorial and thus will not be further discussed.

Clinical presentation of the patient

A 22-year-old female was walking with friends in a park, when she collapsed, began to have difficulty in speaking, which was accompanied by the appearance of a partially swollen tongue, stridor, nausea, vomiting and the rapid development of pinpoint pupils. One of her friends believed they saw a spider over her left eye. The patient, as well as her friend, speculated whether she was experiencing an allergic reaction to a possible spider bite. An ambulance was called and on arrival they found the patient sitting up with assistance, unable to speak, with swelling of her tongue. The EMTs were advised of the evolution of clinical symptoms and signs, and of the spider over the patients left eye. The patient soon became unconscious. The EMTs took the patients vital signs, which were normal, however, they noted she had pinpoint pupils. Suspecting the patient was experiencing anaphylaxis they immediately gave the patient three doses of epinephrine, 3mg per dose, subcutaneously, by way of an EpiPen over the space of 15 to 20minutes, along with a dose each of Ventolin and Benadryl. The patient did not respond, remaining unconscious with pinpoint pupils.

The patient was transported to the hospital, where on arrival her blood pressure was noted to be 174/98 with a pulse of 113. It should be noted a systolic BP of 180 or above is considered to be within the 'hypertensive crisis range' and requires immediate treatment. The patient was described as being unresponsive, with pupils measuring 2mm in diameter, non-reactive and with no corneal reflex. She was moaning, breathing spontaneously, not following commands nor moving legs to painful stimuli. Ten minutes after arrival another blood pressure reading showed her blood pressure to be 174/89. A CT scan of the head revealed a pontine hemorrhage (bleeding into the brainstem) from a arteriovenous malformation (AV malformation)

Discussion

It was the initial impression by those who were with the patient, as well as the EMTs, she was experiencing an anaphylactic reaction to a spider bite. Although it is true, the most common allergens causing systemic anaphylactic reactions include drugs, peanuts, tree nuts, insect venoms, foods, radiocontrast media, allergen immunotherapy injections, and latex. In addition, Hymenopteran (winged insects, such as sawflies, wasps, bees and ants) stings alone are responsible for most arthropod related deaths and usually result from the development of hypersensitivity following repeated exposure to venom. The question that needs to be asked is what types of reactions do spider bites in North America cause. Spiders belong to the order Araneae, which are airbreathing arthropods that have eight legs and chelicerae with fangs that inject venom. There are two spiders of immediate concern in North America, The black widow spider, Latrodectus mactans, and the brown recluse spider, the most famous of which is Loxosceles reclusa.

The black widow spiders most commonly are found in woodpiles, with their bites commonly being to the hands. The toxins they release in their bite are referred to as latrotoxins. These toxins act by increasing intracellular calcium concentrations, depolarizing neurons, and stimulating uncontrolled release of neurotransmitters. Symptoms include local pain as well as systemic symptoms suggestive of an acute surgical abdomen. However, none of these features were seen in this patient. Also, the bite of a black widow spider does not cause an anaphylactic type of reaction. Brown recluse spiders are most commonly found in the South Central United States, from Tennessee and Missouri to Oklahoma and Texas. They are also found in Canada. However, they are not typically found outside, but rather they like to hide dry, undisturbed places, such as baseboards, cellars, furnaces, behind pictures and in infrequently worn shoes. Also, these spiders are very shy thus, bites from these spiders are uncommon. When bites do occur they release two toxins, sphingomyelinase D and hyaluronidase. These toxins cause a localized pyoderma gangrenosum, which is a localized necrosis of the skin and underlying subcutaneous tissues along with a rash, nausea, fever and even death. They may also cause other systemic reactions, which include disseminated intravascular coagulation and Coombs'-positive hemolytic anemia. Except for nausea, none of these features were seen in this patient. The bite of these spiders do not cause an anaphylactic type of reaction.

What we need to ask was the clinical presentation of the patient consistent medically with an anaphylactic reaction? What provides you with some insight into the answer to this question is the initial vital signs taken by the EMTs, showed them to be normal. Those who are experiencing an anaphylactic reaction typically present with as an anxious patient who has an increased heart rate and respiratory rate, neither of which this patient showed. These clinical presentations are followed by hypotension, urticaria (hives), pruritus (itching), and angioedema. Along with these manifestations, bronchconstriction causing wheezing and cyanosis develop, along with laryngeal edema, which in turn causes hoarseness and stridor. None of these clinical manifestations were present in this patient except for the perception the patient had swelling of the tongue due to angioedema, had evidence of stridor and she was unable to talk. Although, angioedema can be a cause of swelling of the tongue, what this patient experienced was unilateral swelling of her tongue. Unilateral swelling of the tongue suggest a cranial nerve injury to the hypoglossal nerve (CNS12). In this patient we have a causation of such an injury in the form of a pontine hemorrhage due to a bleeding AV malformation. What she experienced is referred to as a hypoglossal nerve palsy. The important point to remember, hypoglossal palsy only involves one side of the tongue in the distribution of the hypoglossal nerve involved, whereas angioedema, if it involves the tongue, will typically involve the whole

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or part of the tongue in a patchy, asymmetrical fashion. It typically does not involve part of the tongue in the distribution of CNS12. The other important point to remember, is despite the patient receiving 3 doses of epinephrine, her pupils instead of dilating, remained pinpoint, which also suggest a pontine lesion.

Regarding wheezing and stridor, it is true these are clinical features of bronchconstriction and laryngeal edema respectively, and are often seen in anaphylaxis. This patient had no evidence of wheezing, but did have stridor. It is true, laryngeal edema with or without involvement of the vocal cords can cause stridor. However, there are other causes of vocal cord dysfunction, most especially in a patient who is unconscious with pinpoint pupils, no corneal reflex, does not move their extremities to painful stimuli and has normal vital signs when she is initially seen. One of the causes of vocal cord dysfunction is brainstem compression, which involves the Glossopharyngeal and spinal accessory nerves. This patient suffered brainstem compression due to bleeding from a ruptured AV malformation. The patient was given three doses of epinephrine subcutaneously. Epinephrine is one of the most potent vasopressor drugs known. It is a potent stimulant of both α and β adrenergic receptors. Particularly prominent are the actions on the heart and on vascular and other smooth muscle. Regarding its effect on vascular smooth muscle, epinephrine is one of the most powerful vasopressor (vasoconstrictor) drugs. Thus, the patient's blood pressure will be substantially increased. This patient's blood pressure on arrival at the hospital was initially 174/98 and 10minutes later 174/89, both clearly within the hypertensive crisis range. Another point to remember is one of the known side effects of epinephrine is cerebral hemorrhage due to the sharp rise in blood pressure. Epinephrines affect on the heart is such that it causes the heart to contract strongly. This results in both increasing the end-diastolic volume and decreasing the end-systolic volume, which results in the stroke volume output being increased to more than double. Thus, if there is an already existing intracranial bleed, the quantity of blood expressed in that intracranial bleed is going to be increased substantially, which is not something a patient with a bleeding AV malformation needs. Another effect of epinephrine is it affects the innervation of the iris and ciliary muscles of the eyes, causing dilation of the pupils. It is important to remember, even after three doses of epinephrine, this patients pupils remained pinpoint. The diameter of the pupils is determined by the balance of innervation between the constricting sphincter and radially arranged dilator muscles of the iris, the sphincter muscle playing a major role in the light response. The pupillocostrictor (parasympathetic) fibers arise in the Edinger-Westphal nucleus in the high midbrain, join the third cranial (oculomotor) nerve, and synapse in the ciliary ganglion, which lies in the posterior part of the orbit The postganglionic fibers then enter the globe via the short ciliary nerves; approximately 3percent of the fibers innervate the sphincter pupillae and 97percent the ciliary body, which is primarily responsible for accommodative constriction of the pupil. The pupillodilator (sympathetic) fibers arise in the posterolateral part of the hypothalamus and descend, uncrossed, in the lateral tegmentum of the midbrain, pons, medulla, and cervical spinal cord to the eighth cervical and first and second thoracic segments, where they synapse with lateral horn neurons. The latter give rise to preganglionic fibers, most of which leave the cord by the second ventral thoracic root and proceed through the stellate ganglion to synapse in the superior cervical ganglion. The postganglionic fibers course along the internal carotid artery and traverse the cavernous sinus, where they join the first division of the trigeminal nerve, finally reaching the eye as the long ciliary nerve that innervates the dilator muscle of the iris. What the patient presented with was significant bilateral constriction of the pupils (miosis), which is commonly seen with pontine lesions, presumably because of interruption of the pupillodilator fibers, thus allowing no impediment to the pupilloconstrictor fibers, the exact mechanism however is not clear. Hence, failure of the pupils to dilate should have suggested to the EMT an alternate diagnosis to anaphylaxis.

Conclusion

The clinical presentation of this patient is such, she did not have anaphylactic shock due to a spider bite, but rather an unfortunate pontine hemorrhage due to an AV malformation of her brainstem. Tragically, the EMT's lack of knowledge of what spider bites produce clinically, as well as his lack of understanding of the clinical presentation of anaphylaxis, lead to an unfortunate line of treatment, which had catastrophiccon sequences for the patient. What we need to ask, does laboratory technology have the potential to serve as a valuable tool to help the EMT make a better clinical decision? Could it have given him guidance as to the underlying pathophysiologic process involving the patient and perhaps prevented a misguided line of treatment? If we had a laboratory kit, which the EMT could have used on site, that would have given him some information as to the level of histamine or total tryptase, it may have prevented the line of treatment he decided on. We as laboratorians must look for ways in which the laboratory sciences can aid our clinical colleagues to provide the very best of medical care, for it is in all of our interest. What we must remember, that 22-year-old-woman could have been any one of us.

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Conflict of interest

The author declares no conflict of interest.

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