Review Article

Innate Immunity

Innate Immunity
2018, Vol. 24(8) 466–479

DOI: 10.1177/1753425918808008
journals.sagepub.com/home/ini
SAGE

Whatever happened to the Shwartzman phenomenon?

Abdullah B Chahin[1], Jason M Opal[2] and Steven M Opal[3]

Abstract

Ninety years ago, Gregory Shwartzman first reported an unusual discovery following the intradermal injection of sterile culture filtrates from principally Gram-negative strains from bacteria into normal rabbits. If this priming dose was followed in 24 h by a second intravenous challenge (the provocative dose) from same culture filtrate, dermal necrosis at the first injection site would regularly occur. This peculiar, but highly reproducible, event fascinated the microbiologists, hematologists, and immunologists of the time, who set out to determine the mechanisms that underlie the pathogenesis of this reaction. The speed of this reaction seemed to rule out an adaptive, humoral, immune response as its cause. Histopathologic material from within the necrotic center revealed fibrinoid, thrombo-hemorrhagic necrosis within small arterioles and capillaries in the micro-circulation. These pathologic features bore a striking resemblance to a more generalized coagulopathic phenomenon following two repeated endotoxin injections described 4 yr earlier by Sanarelli. This reaction came to be known as the generalized Shwartzman phenomenon, while the dermal reaction was named the localized or dermal Shwartzman reaction. A third category was later added, called the single organ or monovisceral form of the Shwartzman phenomenon. The occasional occurrence of typical pathological features of the generalized Shwartzman reaction limited to a single organ is notable in many well-known clinical events (e.g., hyper-acute kidney transplant rejection, fulminant hepatic necrosis, or adrenal apoplexy in Waterhouse-Fredrickson syndrome). We will briefly review the history and the significant insights gained from understanding this phenomenon regarding the circuitry and control mechanisms responsible for disseminated intravascular coagulation, the vasculopathy and the immunopathy of sepsis.

Keywords

Shwartzman phenomenon, endotoxin priming, Sanarelli-Shwartzman reaction, endotoxin tolerance, purpura fulminans, septic shock, disseminated intravascular coagulation

Date Received: 6 July 2018; revised: 12 September 2018; accepted: 27 September 2018

Introduction

Pick up any book of animal or human pathology and you will invariably find a section devoted to the generalized Shwartzman reaction or phenomenon. The phenomenon connotes a hypersensitive innate immune response accompanied by an acute small vessel vasculopathy in association with diffuse intravascular coagulopathy (DIC).[1] Pathologists will immediately recognize the characteristic histopathologic features and predict the likely clinical outcome. A rapidly fatal septic shock syndrome with scattered dermal necrosis will develop from *purpura fulminans* following bloodstream infection by *Neisseria meningitidis*. Yet, the term "Shwartzman phenomenon" seems to have fallen out of common parlance over the years, even among clinicians who take care of such patients. Does this nearly century-old observation have any residual relevance in modern medicine? Perhaps it is the current

[1]Infectious Disease Service and Critical Care Division, Memorial Hospital of Rhode Island and the Alpert Medical School of Brown University, Providence, USA
[2]Department of History and Classical Studies, McGill University, Montreal, Canada
[3]The Infectious Disease Division, Rhode Island Hospital and the Alpert Medical School of Brown University, Providence, USA

Corresponding author:
Steven M. Opal, Ocean State Clinical Coordinating Center of RIH, 1 Virginia Avenue, Suite 105, Providence, RI 02905, USA.
Email: Steven_Opal@brown.edu

distaste for the use of eponyms in medical education or that the term has simply been subsumed by other broad categories such as systemic inflammatory states, disseminated intra vascular coagulation (DIC), septic shock, *purpura fulminans*, etc.

We will argue herein that the term has a rather specialized and unique meaning that is worth preserving. Endotoxin sensitization events followed by a provocative second event (the "two hit" model) still occurs in a subset of acutely ill patients. If a priming inflammatory stress is followed with 24–48 h later by a provocative event, the final result can be amplified leading to potentially devastating clinical consequences.

The early history of the Shwartzman phenomenon

Gregory Shwartzman (1896–1965) was born in Odessa, Russia, and received his medical degree in Brussels, Belgium. He did his post-doctoral training at the Lister Institute in London. He then immigrated to the United States in 1923 and began his work in bacteriology at the Mt. Sinai Hospital in New York City. He became the director of the Department of Bacteriology in 1926 and made his seminal discovery on what we now call the dermal or local Shwartzman phenomenon over the next 2 yr with his first publication in 1928.[2]

Using sterile culture filtrates of the Gram-negative bacterium *Bacillus* (a.k.a.) *Salmonella typhosus,* he repeatedly demonstrated in several hundred rabbits that an intradermal injection of the culture filtrate as a preparatory injection, followed by a second provocative dose of the same culture filtrate intravenously 24 h later, induced a localized area of severe, hemorrhagic necrosis at the first injection site.[2] The timing between injections was critical; if the provocative challenge dose was too short (<2 h) or too long (>48 h) the dermal reaction did not occur. He noted that the same stereotypical reaction was highly reproducible in most rabbits. However, 22% of the rabbits failed to respond at all and were refractory to each attempt. This was not widely recognized at the time, but this is likely an example of a related phenomenon known as endotoxin tolerance described decades earlier.[3]

Shwartzman experimented with similar preparations with culture filtrates from streptococcal species and failed to duplicate any dermal reactions implicating the primary, but not exclusive, role of Gram-negative cell wall constituents (LPS) to induce the phenomenon. Endotoxin "tolerance" (or more correctly endotoxin "reprogramming") induces many counteracting effects which can block some of the hypersensitivity features displayed in the Shwartzman reaction.[2–6]

Four years before Shwartzman's first publication, an Italian investigator named Giuseppe Sanarelli described similar but more generalized pathological findings in rabbits given a sensitizing dose intravenously, followed by a second provocative intravenous dose of culture filtrates from *Vibrio cholerae*.[6] These sterile preparations were generated by passing culture supernatants over a submicron filter to trap any remaining viable bacteria.[6,7] This generalized syndrome of intravascular coagulation and microvascular plugging became known as the generalized Shwartzman-like reaction (or phenomenon). Intravascular clotting was an essential part of the dermal and generalized reaction requiring clotting substrates and adequate amounts of fibrinogen.[8]

The Arthus reaction, endotoxin tolerance, and the Shwartzman phenomenon

What initially attracted Dr. Shwartzman's attention, and those of his contemporaries, was that the skin lesions created by this reaction were histologically similar to another recently described reaction call the Arthus phenomenon.[9] The Arthus reaction was an immune-mediated, hypersensitivity angiitis, involving small arterioles, capillaries, and venules. The lesions showed fibrinoid necrosis with intravascular thrombosis with abundant platelets and granulocytes. Antibody-antigen complexes and complement deposits were readily detectable in the Arthus reaction but not in the Shwartzman reaction.

While Shwartzman's discovery shared many of same histopathologic features, it differed strikingly in several crucial aspects. The Arthus reaction is highly specific with definable Ab complexes, which took weeks to develop and persisted for years. The localized Shwartzman reaction was non-specific, as substituting a second Gram-negative species culture filtrate was sufficient to cause dermal necrosis. The reaction occurred over too short a time for Ab formation and disappeared in a few weeks.[3,7]

Details of cellular immunology of the time did not clearly separate innate immunity from acquired immunity, but chronic immune reactions (lymphocytes), acute phagocytic cellular responses and humoral immunity with Abs and elements of the complement system were reasonably well understood. What Shwartzman had described was something new for endotoxin researchers to study and contrast with the observations of a competing process now referred to as endotoxin tolerance or reprogramming.[10–13]

Endotoxin tolerance was described more than a century ago by Centanni and colleagues during their efforts to use pyrogenic bacterial culture sterile filtrates

as therapeutic agents against neoplasms and certain refractory infections.[3,4] Fever was generated by injections of sterile culture filtrates of *Serratia* spp. and other bacterial filtrates demonstrated some initial therapeutic effects. However, after several therapeutic doses, the pyrogenic filtrates lost the ability to cause fever and the clinical benefits of treatment despite raising the dose 10-fold or more.[3] Endotoxin tolerance is now appreciated to occur at the transcriptional level where initial pro-inflammatory responses become tolerized over time into a state of systemic inflammatory deactivation, with some preservation anti-microbial defenses. This topic has been recently reviewed.[10–13]

Coley's toxin and the local Shwartzman reaction as an anti-neoplastic therapy

In the 1890s, William Coley, a surgical oncologist from New York City, developed what was then called Coley's toxins.[14] He experimented with this material to induce necrosis and radical cures for patients with advanced malignancies, particularly sarcomas. He and others had had observed that patients with inoperable malignant tumors would occasionally exhibit marked regression of the tumor size if it happened to be in close proximity to an infected site. It was thought that this "collateral damage" to tumors from local inflammation could be harnessed clinically by carefully placing infectious foci adjacent to or inside a neoplastic mass.

He pursued this finding further by using live injections of *Streptococcus pyogenes* from other hospitalized patients with active facial erysipelas. He would inject the bacteria directly into tumors daily for weeks attempting to induce tumor regression. For safety reasons, he later converted to using sterile culture filtrates derived from *S. pyogenes* and the Gram-negative bacillus *Serratia marcescens*. He regularly noted that he would have to increase the dose of toxic combination over time to achieve the desired effect of tumor regression. He called it "second generation" dosing, but he was actually independently confirming the process of endotoxin tolerance.[3]

Coley's limited success with tumor regression was later attributed to the endotoxin found in Coley's toxin, which markedly induced TNF, IL-1, and perhaps other lethal cytokines such as IL-12.[14,15] Shwartzman himself tried to replicate some of Coley's work by showing that the generalized Shwartzman reaction could elicit hemorrhagic necrosis and regression of transplanted sarcoma tumors in Guinea pigs, rats, and mice.[16] Further studies into the cellular and molecular mechanisms responsible for the generalized Shwartzman reaction and its opposing effects to endotoxin reprogramming have expanded the understanding of coagulation and innate immune responses to neoplasms, inflammation and thrombosis. These studies continue to the present day (see Table 1).[9–12,17–26]

These early studies into immune activators to treat cancer were gradually abandoned as the results were highly variable and often quite toxic. However, the strategy of treating neoplastic diseases with immune adjuvants has recently seen enormous gains in interest with the success of monoclonal antibodies against check-point inhibitors such as PD1.[27] Patients with previously considered inoperable tumors with little hope have seen some remarkable recoveries by administration of these precise immune T cell activators.

Contributions of the local Shwartzman phenomenon to experimental biology

Numerous discoveries relating to activators and inhibitors of endotoxin activity were aided by the availability of the simple and reproducibility of the dermal Shwartzman reaction. Intradermal injection of the pro-inflammatory cytokines IL- I and TNF,[15] and IFN-γ and IL-15 were found to be an effective substitute for the intradermal injection of endotoxin in the local Shwartzman reaction.[15–26] Exposure to those cytokines in high dose can induce endothelial cells to become thrombogenic and can induce the expression of cell adhesion molecules on endothelial cells, increasing the adherence of leukocytes to the endothelial cells.[28–30] However, the provocative intravenous injection of LPS can also be substituted by a number of agents, but most of them are not as effective as LPS.

Perhaps the most enduring value of the dermal Shwartzman phenomenon has been its utility as a highly sensitive and specific biomarker for the biochemical and biophysical requirements for endotoxicity. Decades of laboratory investigation into structural immunology of LPS was based upon the dermal Shwartzman reaction as an accurate bio-read out for endotoxin. Through a detailed interrogation of the physiochemical requirements for endotoxicity, the length, number, size and arrangement of the fatty acids that make up lipid A in relationship with MD2 (myeloid differentiation factor 2) and TLR4 was predicted with great accuracy .[31,32] It was discovered that hexa-acyl, di-phosphorylated disaccharides would be stimulatory agonists, while tetra-acyl, mono-phosphorylated molecules would be antagonists and function as inhibitors of LPS signaling. The final three-dimensional crystal structure of the LPS-MD2-TLR4 now explains many of the predicted structural requirements for full endotoxicity using the dermal Shwartzman as a reliable, *in vivo* guide post.[33]

Table 1. Contrasts and comparisons between the generalized Shwartzman-like phenomenon and endotoxin tolerance.

	The dermal or generalized Shwartzman-like reaction ("two hit" model)[11–26,56–58,65–68]	Endotoxin tolerance (LPS reprogramming)[11–26,56–58,65–68]
Overall effect	Endotoxin sensitization	Endotoxin de-sensitization
Duration of the effect	Short (<48 hr)	Long; Begins within 24 h and lasts for up to 21 d
Serotype specificity	None, could substitute other LPS types but not seen with Gram-positive bacteria	None, serotype-independent
Innate or acquired cellular or humoral immune response	Innate immunity and coagulopathy	Innate response driven primarily by myeloid cells
Need for complement	Yes	No
Inhibition by heparin or salicylates	No effect	No effect
Requires neutrophils, platelets, and fibrinogen	Yes	No
Glucocorticoid pre-treatment allowed a single dose of endotoxin to induce the reaction	Yes	No
TNF, IL-1 or the combination can substitute for endotoxin	Yes	No
IL-12, IL-15 and/or IFN-γ can substitute for endotoxin	Yes	No
Homologous Abs, but not heterogeneous Abs, block reaction	Yes	Unknown

How did Dr. Sanarelli contribute to the Sanarelli-Shwartzman phenomenon?

It should be noted that Shwartzman described the localized dermal form of this phenomenon. The generalized Shwartzman-like reaction was actually discovered and initially reported 4 yr earlier in 1924 by the brilliant, but highly controversial, physician-investigator named Giuseppe Sanarelli (1865–1940).[6] The generalized reaction is sometimes referred to in the literature as the Sanarelli-Shwartzman reaction in honor of the major contributions made to this research effort by Dr. Sanarelli. Sanarelli was an Italian researcher who was a well-trained bacteriologist studying in established microbiology research laboratories in Munich, Germany, and then at the Pasteur institute. He listed Louis Pasteur and Elie Metchnikoff among his mentors. He became an independent investigator in 1895 and accepted an offer to move to Montevideo, Uruguay to establish his own laboratory.

The late 19th century was the pinnacle of the age of the "microbe hunters",[34,35] and Sanarelli wanted to be part of it. Pasteur had proven the germ theory of disease, and he had demonstrated that attenuation of pathogens in the laboratory made it possible to develop an antiserum or even a vaccine to eliminate many infectious diseases. Many new bacterial pathogens were being discovered and correlated with common infectious diseases of humankind. Koch uncovered evidence that tuberculosis was a transmissible infectious disease in 1882, and is credited with first isolating *Vibrio cholerae* in 1884. He also added much needed clarity and order to the process of establishing claims of new microbial disease causation by following Koch's famous postulates.[35]

By the end of the 1890s, a major, unsolved mystery remained in discovering the possible infectious cause of the highly lethal disease called Yellow Fever. This devastating disease carried a case-fatality rate nearly 33–50%, and still does today. Referred to as the "scourge of the tropics" or "stranger's disease," it often struck new workers and recent arrivals into regions with endemic Yellow Fever. The disease brought headache, confusion, weakness, general malaise, relative bradycardia, often followed by "black vomit" (hematemesis), deep yellowing of the skin (jaundice from liver necrosis), and death.[36]

Yellow Fever epidemics first took hold in the New World as slave workers from Africa began to fill the void of farmers and laborers needed in Central, South, and North America.[37,38] Young, healthy people were less likely to die from Yellow Fever but no age group was spared. Fortunately, survivors were rendered immune to subsequent infection. Epidemics occurred through the coastal communities from Brazil as far North as Pennsylvania. Outbreaks in Philadelphia (then the US capital) in 1793 and 1798 nearly crippled the young nation's administration and intensified the violent partisan conflicts of the day.[38] Whoever discovered the causative agent of Yellow Fever and its

primary mode of transmission would be an instant, international folk hero indeed.

Many theories were postulated to explain the cause of the illness ranging from bad water, miasma theory (airborne mists and contaminated, dirty, living conditions), fomites (particularly bed cloths and linens) mosquitos, fungi, bacteria, or tiny filterable agents.[38–40] Filterable agents were recognized at the time as viruses that could pass through a submicron filter that would effectively trap almost all bacteria expressing a cell wall.

The race to discover the cause of Yellow Fever was on. This open and quite public challenge pitted South and Central American physician-scientists against each other and a number of European investigators interesting in expanding their influence and prestige within each host countries' respective empires.[37] Even the US military took an interest as Yellow Fever was a major non-combat killer of soldiers in the Spanish-American War (1898–1900). Yellow Fever was also a formidable impediment to American designs to finish building the Panama Canal after the French had abandoned the effort 20 yr earlier.[38] The race to discover the cause of Yellow Fever attracted much of the public's imagination and their attention.

Sanarelli was a bacteriologist at heart but his first major contribution to science was correctly recognizing the viral cause of an entirely new neoplastic disease first detected in imported European rabbits into South America in 1896. Rabbits were the favored laboratory animals for raising antisera by microbiologists, including Sanarelli's own laboratory. He was disturbed to find that imported rabbits quickly succumbed to this new illness, which was contagious in European rabbits but not in the local, tropical rabbits found in South America.[41]

He also recognized that European rabbits housed in cages in the outdoors rapidly developed the disease while indoor rabbits caged inside were largely spared from the disease. He correctly speculated that outdoor rabbits would be exposed to mosquitos and this must be the way the lethal disease was transmitted. Autopsies revealed the disease to manifest as multiple tumors in multiple organs and he called the disease myxomatosis.[41] He discovered it was caused by a filterable agent, later to be confirmed as an oncogenic, lethal poxvirus. Local strains of rabbits were apparently immune, while imported rabbits were highly susceptible.[41–43] This same virus was later used in Australia as an abortive attempt to biologically control the massive populations of European rabbits that arose after their introduction into Australia.[44] Sanarelli was widely and rightfully acknowledged as a gifted microbiologist for this major discovery. He then set out to find the microbial cause of Yellow Fever.

In 1897, he mistakenly announced that he had found the bacterial cause of Yellow Fever in the blood of its victims.[40] He also announced that he had developed a curative intra-venous antiserum that he considered "tellement simple et tellement sur" (so simple and so safe). His claim was immediately taken seriously as he was a respected microbiologist, competent in the latest techniques of sterility and familiar with careful scientific methods. He called the pathogen *Bacillus icteroides*. He then sought to prove Koch's postulates and he appropriately shared his pathogen and his data with other competing laboratories. He even designed clinical trials to test an antiserum he had developed to defend against his newly discovered, causative bacterium. Other laboratories tried to replicate his results with mixed and contradictory results, and began to question the validity of his claims.[40,45,46]

Around this time, the Yellow Fever Commission from the US showed up to try to bring some order to the chaotic situation.[38,47] General George Sternberg reviewed the Sanarelli data and found fault in his methods, sterile technique, and the analytic methods used in his early clinical trials with antisera. Within 2 yr the American mission under the capable command of Walter Reed, and with assistance from a Cuban investigator named Carlos Finlay, confirmed that Yellow Fever was a mosquito-borne, viral disease transmitted by *Aedes aegypti* (previously called *Stegamyia faciens*) in 1900.[48] Sanarelli probably misinterpreted his own data and the pathogen he thought caused Yellow Fever was likely a contaminating strain of *Salmonella* spp. known as *S. cholerae suis* that causes hog cholera.[48,49]

Sanarelli compounded his difficulties by trying to convince others he had discovered a bacterial cause of Yellow Fever. He injected his bacterial pathogen intravenously to cause disease in uninformed, human "volunteers," and then proceeded to attempt to rescue them with his experimental anti-serum.[49] Details are sketchy but some of these participants were likely prisoners from a nearby institutional outbreak of suspected Yellow Fever. As Dr. Sanarelli noted, the prison epidemic was "un hasard vraiment heureux" (a stroke of good luck) for acquiring study subjects for his human experiments.[40,49] Apparently, three of the first five study subjects reportedly died from the experiment. This fiasco did, in fact, demonstrate that Sanarelli had discovered a potentially lethal, bloodstream, bacterial pathogen, but it was not the cause of Yellow Fever.

This rather callous human experiment dismayed the medical community and tarnished the reputation of Dr. Sanarelli. No formal international standards for human subjects in medical experimentation existed at the time and the Nuremburg trials of Nazi doctors and

the Helsinki accords were more than a half century away.[50,51] Nonetheless, Sanarelli was roundly criticized by anti-vivisectionists and his fellow researchers as well. Sir William Osler is reported to have said publically, "To deliberately inject a poison of known high degree of virulency into a human being, unless you obtain that man's sanction, is not ridiculous, it's criminal".[51]

It should be noted that a generally accepted code of medical ethics for human experimentation did not exist in the 18th and 19th centuries. Edward Jenner performed probably the most important medical experiment in history by demonstrating that vaccinia (cowpox) from the hands of milk maids, if delivered by skin incisions in young children, could protect against variola infection (small pox).[52] These were uncontrolled experiments done in children, without any evidence of written consent, and followed by actual smallpox virus challenge inoculations. Fortunately, the experiment was successful, and the rest is history. Risky medical experiments, without proper informed consent, continued to be performed on vulnerable subjects such as convicted prisoners and mentally impaired patients in the US up until the mid-1900s—a practice now universally considered unacceptable.[53,54]

Partly in response to this medical research scandal, the Walter Reed commission went to great lengths to provide a detailed, well written, informed consent for all human experiments needed to determine the cause and method of transmissibility of Yellow Fever.[46–48] They followed an egalitarian ethos prevalent at the time that championed the idea; "Before you perform experiments on human subjects, you should be willing to perform the same experiment on yourself first." The four lead investigators of the Yellow Fever commission were Walter Reed, James Carroll, Jesse Lazear, and Aristides Agramonte. Dr. Agramonte had already survived Yellow Fever years earlier and was thus immune to reinfection. Carroll allowed infected mosquitos to feed upon his exposed skin. Within a week he was deathly ill but survived. Lazear was next to roll up his sleeve to infected mosquitos and promptly died from the experiment. Walter Reed wisely elected to halt further testing on fellow researchers who had sacrificed enough.[48]

Sanarelli never fully recovered from his misadventures with finding the elusive Yellow Fever pathogen.[49] He spent the rest of his career working on the pathogenesis of *Vibrio cholerae* in experimental animals.[56] Through a detailed and careful set of timed experiments, he proved that endotoxin-laden, culture filtrates from *V. cholerae* could cause a thrombotic diathesis both locally within nutrient blood vessels within the gastrointestinal tract, and systemically in distant organs such as the kidney and spleen. He could reproduce intravascular clotting of mesenteric blood vessels using culture filtrates given intravenously if separated by 24 hr. This effect was independent of the well-known, diarrhea-causing cholera exotoxin.

Importantly, he showed that the same dose of live bacteria vs. killed bacterial intravenous injections of *Vibrio cholerae* was equally lethal in the rabbit model. This was early evidence of the critical role of endotoxin in the pathogenesis of enteric Gram-negative bacterial infections. He published his work in a series of papers from 1919 to 1924.[6,55] These histopathologic findings from the Sanarelli laboratory were notable for their similarities with the localized dermal reaction observed by Shwartzman a few years later in the late 1920s. The combined discoveries are now often referred to simply as the Shwartzman phenomenon or, perhaps more correctly, the Sanarelli-Shwartzman phenomenon.

Surprisingly, neither man referenced the other investigator's work in their initial study reports. It does not appear that they ever actively collaborated with each other in their laboratory projects. It is possible, perhaps even likely, that they never met. Sanarelli's laboratory work was done in Uruguay and in Europe, and he was 31 yr older than Shwartzman. Sanarelli published most of his work in French, but also in the German and the Italian scientific literature. His most important primary publications were all published in French in the *Annales d' Institut Pasteur*.[55]

Pathophysiology of the Shwartzman-like reaction

The generalized Shwartzman reaction is created in experimental animals by injecting two, carefully spaced, sublethal doses of endotoxin. The time interval required between first (preparatory) and second (provocative) dose is usually between 2 and 48 hr. The reaction manifests intravascular "hyaline" or "fibrinoid" deposits, which are immuno-chemically identical to fibrin.[56] The reaction carried out *in vivo* causes deposition of fibrin in kidneys, liver, spleen, and lungs as well.[57] The fibrin deposits gradually disappear from all these organs except the kidneys, which appears after the second (provocative) dose in generalized Shwartzman reaction. Those deposits are not only a result of blood clotting and intravascular blood clotting, but also of inhibition of fibrinolysis.[58] This and other variants of the "two hit" model of sepsis are currently in widespread use in research laboratories around the globe, but are seldom referred to as the Shwartzman reaction in the current literature.[60,61]

Studies of sepsis in humans and other species consistently reveal that innate immune activation and

disturbances in coagulation to be inseparably linked, with each acting as positive feedback for activation of the other.[59,60] Evidence of diffuse coagulation activity with thrombocytopenia is highly predictive of mortality in sepsis.[61,62] Activation of the coagulation system is thought to be the primary event that triggers Shwartzman phenomenon, leading to a consumptive coagulopathy of the microvasculature, which can be localized or generalized, acute, subacute, or chronic. Intravascular coagulation activation likely contributes to multisystem organ dysfunction syndrome (MODS).[63,64]

Necrotizing hemorrhagic tissue reaction is the hallmark of the Shwartzman-like reaction. Microthrombi composed of platelets and leukocytes accumulate in the vessels after the exposure to endotoxins. The Shwartzman reaction depends on integrity of the clotting system, on platelets and on polymorphonuclear leukocytes, and the complement system.[19–23] Intravascular activation of complement triggers the release of anaphylatoxins such as C3a and C5a into the circulation seem to play a major role in the propagation of Shwartzman reaction. The cleavage products activate neutrophils and platelets, causing them to aggregate and adhere to vascular endothelium. This results in the occlusion of small vessels and release toxic mediators.[14,21,64] In practice, local and generalized Shwartzman reactions are models of thrombohemorrhagic skin necrosis and DIC, respectively.

The robustness of the immune response, and the number of circulating leukocytes at the time of preparing (i.e., the first dose of toxin) and provoking (i.e., the second dose of toxin) seem to have a significant impact on the development of generalized Shwartzman reaction. A study by Dr. Lewis Thomas found that the generalized Shwartzman reaction, produced by two injections of meningococcal toxin in rabbits, was aggravated by the administration of cortisone and corticotropin (ACTH).[19–21] In contrast, when leukopenia was produced before the preparing injection of toxin (using treatment with nitrogen mustard) the generalized Shwartzman reaction was inhibited.[21] However, when the femoral bone marrow was shielded from the action of nitrogen mustard and leukopenia was prevented, no inhibition of the generalized Shwartzman phenomenon was demonstrable. A summary of the advances in understanding the interactions between the coagulation system and innate immunity, differences between endotoxin priming and endotoxin tolerance are listed in Table 1.[11–26,65–67]

Univisceral Shwartzman-like reaction

Local Shwartzman reaction can be induced many different tissues and is not limited to the skin.[68,69] The reaction can be induced in the lung by repetitive intratracheal administration of an inoculum.[70] Acute hepatic necrosis can also be produced by a local Shwartzman-like reaction. The locality of this reaction is related to its "univiscerality", where only a single organ is targeted.[70,71] For that reason, a distinct type of Shwartzman-like reaction, called "Univisceral Shwartzman Reaction", has been proposed. Much of the organ damage accompanied by sepsis, such as acute liver necrosis, Waterhouse-Friderichsen's syndrome,[71] hemolytic-uremic syndrome, idiopathic pulmonary hemorrhage, acute pancreatitis, acute pituitary necrosis, and pseudomembranous colitis[72,73] all seem to have features suggestive of this type of single, organ-specific, Shwartzman-like reaction with focal intravascular coagulation.[74]

Ischemia, although a major contributor to the development of the dermal Shwartzman reaction, might not be the primary cause of the phenomenon. A study to determine the baseline sensitivity of rat skin to ischemia, showed that a period of ischemia was insufficient to cause necrosis. The study suggested that the bacterial infection can sensitize tissues to the effects of ischemia, effectively lowering the threshold to acquiring irreversible cell injury (see Table 2).[75]

The "two-hit" experimental model of sepsis, DIC, and netosis

The two-hit experimental model of sepsis is a variant of the generalized Shwartzman-like reaction and is accompanied by pronounced changes in blood clotting and fibrinolytic systems. In addition, circulating neutrophils were found to rapidly decrease shortly after injection, along with a significant decrease in circulating platelets. The margination of neutrophils in the pulmonary vasculature accounted for most of the trapped neutrophils.[76,77] Netosis, the intravascular generation of neutrophil extracellular traps (NETs), likely contributes to accumulations of platelets and neutrophils within the microcirculation.[62,77] Micro-particles released from damaged endothelial cells and myeloid cells further accelerate the pro-coagulant and proinflammatory actions of intravascular thrombin generation.[59–61] Thrombin generation, activated factor X and tissue factor (TF): FVIIa complexes are all highly inflammatory events via activation of the four human protease activated receptors (PAR) expressed on endothelial cell surfaces (see Figure 2).[62–64]

Ample evidence now exists that demonstrates a wide-ranging cross-talk between hemostasis and inflammation, which is likely implicated in the pathogenesis of organ dysfunction in patients with sepsis.[64] The generalized phenomenon involves disseminated

Table 2. Clinical presentation and mediators of univisceral and general Shwartzman reaction.

	Organs involved	Clinical presentation	Mediators[69–75]
Dermal	Skin	Skin purpura	Intradermal LPS, IL-1, IFN-γ, and TNF
Uni-visceral	One of the following: Lung, liver, kidney, pancreas, colon, pituitary, or adrenal gland	Single organ failure or dysfunction:	Intratracheal or IV endotoxin administration
		- Acute liver necrosis	Hepatotoxins, hepatitis
		- Waterhouse-Friderichsen's syndrome	DIC, adrenal apoplexy
		- Hemolytic Uremic Syndrome	Enterohemorrhagic *Escherichia coli*
		- Idiopathic pulmonary hemorrhage	Unknown, possibly toxins
		- Acute pancreatitis	Chemical or ductal obstruction
		- Acute pituitary necrosis	Severe obstetrical hemorrhage
		- Pseudomembranous colitis	*Clostridium difficile*
Generalized	Two or more of the following: Lung, liver, kidney, pancreas, colon, pituitary, or adrenal gland, bone marrow, blood cells, conjunctiva	MODS	IV administration of endotoxin
		DIC	Septic shock
		HUS	Enterohemorrhagic *E. coli*
		TTP	ADAMTS 13 auto-Ab
		Purpura fulminans	Meningococcemia

HUS: Hemolytic uremic syndrome; MODS multi-organ dysfunction syndrome; DIC disseminated intravascular coagulation; TTP: thrombotic thrombocytopenic purpura

intravascular coagulation (DIC) beginning with upregulation of the TF pathway of hemostasis via endothelial TF exposure to circulating factor VII. The TF: FVIIa complex initiates the TF pathway of clotting. Concomitantly, platelets are activated when surface receptor glycoprotein Ibα encounters excess levels of high molecular mass von-Willebrand Factor polymerized under conditions of shear stress to exposed collagen fibers. Coagulation abnormalities are nearly universal in septic patients and is a key contributor to the development of multiple organ failure.[61,62] Fibrin deposition is not cleared efficiently and it precipitates and remains within vessels (see Figure 1a and b).[78,79]

Fulminant DIC presents in severe sepsis and is manifested in both thrombosis and diffuse hemorrhage. Initiation of coagulation activation and consequent thrombin generation is now believed to be caused by expression of TF on activated monocytes and endothelial cells. This reaction is at a level where it become ineffectually offset by TF pathway inhibitor. At the same time, the protein C system and other endothelial-associated anticoagulant pathways are impaired by pro-inflammatory cytokines.[79] In addition, the removal of fibrin is hindered by inactivation of the endogenous fibrinolytic system. Increased fibrin generation, coupled with its impaired breakdown, lead to deposition of microvascular clots in dermal vessels and throughout the body.[78,79]

The most clinically relevant syndrome in which the generalized Shwartzman-like reaction appears to play a central role is purpura fulminans. The similarities between the generalized Shwartzman reaction and *purpura fulminans* in septic patients were commented upon by Shwartzman and his colleagues in their later writings.[80,81] These findings which lead them to speculate that similar underlying molecular mechanisms were responsible for the tissue injury.[80]

The dermal Shwartzman reaction is histologically indistinguishable from hemorrhagic necrosis of the skin seen in some patients with meningococcemia and septic shock.[82,83] Pathologic and massive activation of coagulation followed by diffuse formation of microthrombus formation which can coalesce into patches of dermal necrosis. *Purpura fulminans* is a tragic clinico-pathologic syndrome that proceeds at a frightening pace in previously healthy children or young adults. Fatalities can result in as little as 24 hr from onset of symptoms. The histopathologic similarities between *purpura fulminans* and the dermal Shwartzman phenomenon suggest that *purpura fulminans* may be the closest clinical illness to what Shwartzman initially described experimentally in 1928.[2]

While *purpura fulminans* is occasionally seen with other Gram-negative bacterial pathogens, *Neisseria meningitidis* is the principal causative microorganism. The mechanisms underlying this well-known association between meningococcemia and overt tissue thrombosis and skin necrosis is now becoming better understood (see Figure 2). Greater adherence of its meningococcal LOS is found in the outer membrane, which has a greater ratio of lipid A content versus polysaccharide content found than most bloodstream enteric Gram-negative pathogens.[82] Experimentally, the

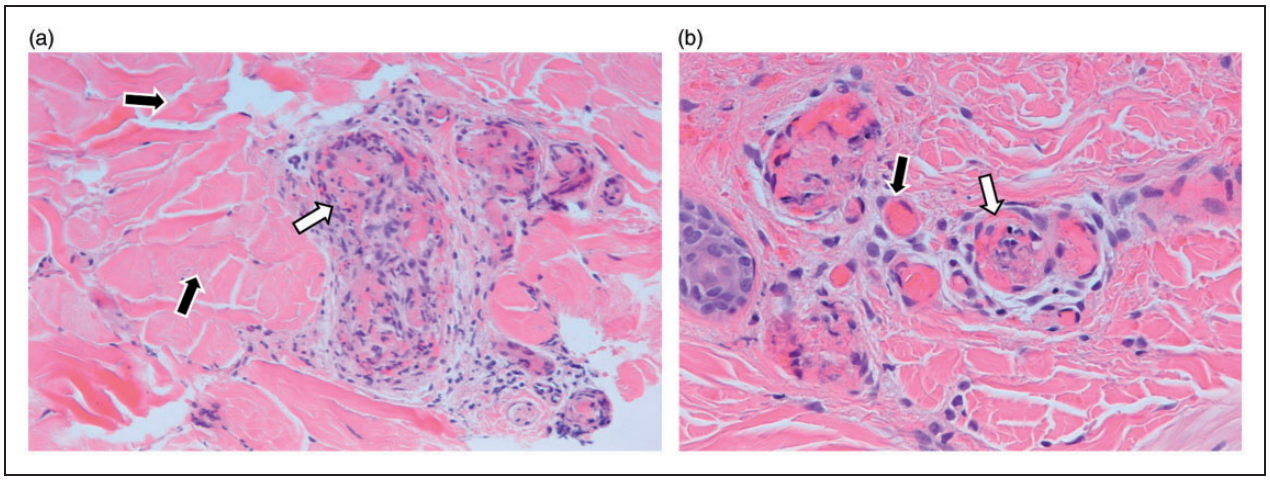

Figure 1 (a) Skin biopsy (20×) showing evidence of dermal necrosis from small vessel obstruction from deposition of fibrinoid material, platelets, and nuclear debris within the capillary lumen in a patient with acute meningococcemia. The black arrows highlight areas of tissue necrosis surrounding obstructed blood vessels. The white arrow shows occluded vessels with fibrinoid material, neutrophil remnants and platelets. (b) Skin biopsy of the same patient at higher magnification (40×); the black arrow shows thrombosis in capillaries with RBCs clogging the vessel lumen; the white arrow shows a damaged vessel wall with swollen endothelial cells with white blood cells and platelets along the vessel lumen with evidence of extravasation of RBCs and dermal necrosis. Histopathology slides are provided courtesy of Gladys Telang.

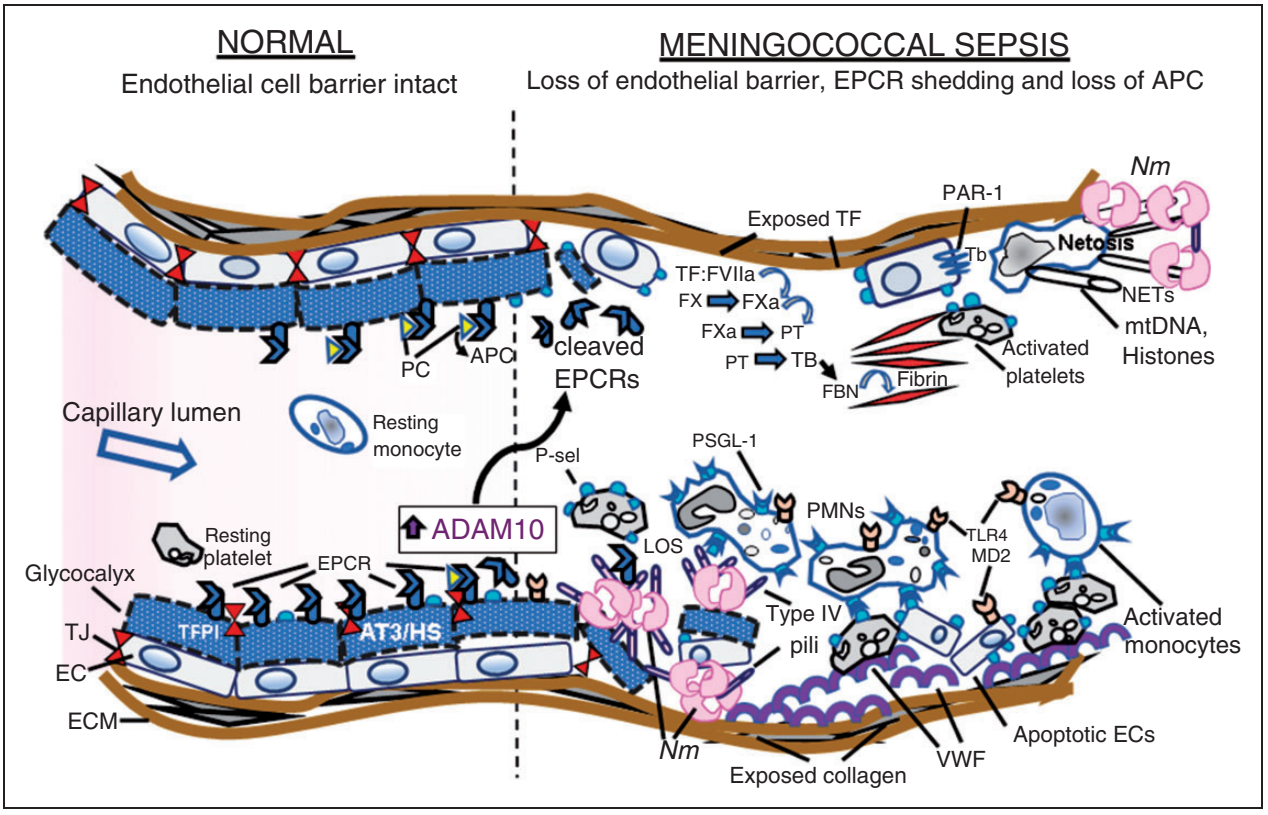

Figure 2 The generalized Shwartzman-like phenomenon during meningococcemia.[77,79,82–85] Induction of ADAM-10 cleaves EPCR, impairs APC formation, and leads to purpura fulminans.
TJ: Tight junctions; EC: endothelial cells; ECM: extracellular matrix; TFPI: tissue factor pathway inhibitor; AT3/HS: anti-thrombin 3/heparan sulfate; EPCR: endothelial protein C receptor; ADAM-10: a disintegrin and metalloprotease-10; PC: protein C; APC: Activated Protein C; P-sel: P-selectin; Nm: *Neisseria meningitidis*; PSGL-1: P-selectin glycoprotein ligand-1; PMNs: polymorphonuclear cells; TF: tissue factor; F: factor; PT: prothrombin; Tb: thrombin; FBN: fibrinogen; MD2: myeloid differentiation factor 2; VWF: von Willebrand's factor; PAR-1: Protease activated receptor-1; NETs: neutrophil extracellular traps; mt DNA: mitochondrial DNA.

Table 3. Pathogens associated with generalized Shwartzman-like reaction with sepsis-induced *purpura fulminans*.

Pathogen	Primary pathogen-inducing factor(s)	Important host factors
Neisseria meningitidis[82–84]	LOS, induce EPCR shedding enzyme	Lack of protective Abs, Complement deficiency, asplenia
Vibrio vulnificus, other *Vibrio* spp.[91–94]	Possible role of cytotoxins	Exposure to salt water through open wounds, liver disease, immune compromise
Aeromonas hydrophilia[95,96]	Unknown	Exposure to fresh water or brackish water through open wounds, immune compromise
Staphylococcus aureus[97]	Superantigens, cytotoxins	Immune compromise
Streptococcus pyogenes[98]	Protective capsules	Lack of protective Abs, asplenia
Streptococcus pneumoniae[99]	Protective capsules	Asplenia, lack of protective Abs
Haemophilus influenzae[98]	Protective capsules	Lack of protective Abs, asplenia
Capnocytophaga canimorsus[100]	Unknown	Asplenia, dog bites, immune compromise
Escherichia coli[101,102]	Possible cytotoxic necrotoxic factors, virulence plasmids	Unknown
Bacillus anthracis[103]	Lethal toxin and edema toxin	Unknown
Israeli spotted fever (*Rickettsia conorii* subspecies *israelensis*)[104]	Unknown but this sub-species carries a high mortality rate	Exposure to infected tick vector

LOS of meningococci resembles rough LPS found in some enteric Gram-negative bacteria, which are incompletely linked to long polysaccharide side chains. The LOS of meningococci are 5–10 times more potent in the dermal Shwartzman reaction but are not more potent in mouse lethality assays.[82]

Meningococcal invasion of the bloodstream leads to binding of the pathogen to the apical surface of endothelial cells within the microcirculation via their type IV pili.[83,84] This event induces endothelial cells to generate a shedding enzyme called ADAM-10 (a disintegrin and metalloprotease-10), which cleaves the endothelial protein C receptor (EPCR).[84,85] This results in the loss of a major feedback inhibitor of ongoing coagulation and endothelial cell damage known as Activated Protein C (APC).[79,85] The generation of APC occurs when the circulating zymogen Protein C is activated by partial proteolysis by thrombin: thrombomodulin complexes. APC is the major inhibitor of intravascular clotting by degradation of the two acceleration factors of the coagulation system FVa and FVIIIa. EPCR: APC complexes on the endothelial cell surface also activate the cytoprotective effects of APC. Both these control mechanisms are lost when meningococci invade and induce EPCR shedding.[84,85]

Infants born with congenital defects in the Protein C pathway rapidly develop *purpura fulminans* within the first few days of life that closely resembles the clinical and pathologic findings in meningococcal *purpura fulminans*.[86] Early treatment in these infants with protein C concentrate will prevent ongoing thrombosis and tissue necrosis testifying to the central role of the Protein C pathway deficiency in these syndromes.

While clinical or autopsy evidence of systemic microthrombi or dermal necrosis is quite rare in patients with septic shock today,[64] biochemical evidence of systemic coagulation activation and platelet activation is almost uniformly present.[59–64] The magnitude of coagulation abnormalities is directly correlated with increasing hospital mortality rates in septic patients.[59,61,79,87] A recent nation-wide registry in Japan found that early treatment with systemic anticoagulation significantly improved overall hospital mortality rates in patients with infection-related disseminated intravascular coagulation.[88] Similar findings have been reported in large clinical cohort studies in the US, where the severity of coagulopathy was directly linked to hospital mortality.[87] Clinical trials with a recombinant human form of soluble thrombomodulin can restore and regulate the APC pathway in sepsis-associated DIC. This regulatory protein or similar proteins might provide a new treatment strategy for sepsis-induced coagulopathy.[88,89]

Summary and conclusions

The discovery of the Sanarelli-Shwartzman phenomenon has taught us a great deal about the interactions between coagulation and inflammation, endotoxin priming and tolerance, and the endothelial membrane interface with clotting and innate immune signaling. The combined effects on the coagulopathy, vasculopathy and immunopathy of septic shock is beginning to take shape. Hopefully, this knowledge will translate

into improved treatments to manage sepsis and other inflammatory states.[90]

Elements of the Shwartzman-like reaction will continue to be useful in the study of two-hit models of sepsis in the animal laboratory. Hopefully, insights provided in the laboratory will benefit in the care of complicated patients who have sustained multiple physiologic insults from infection, trauma, immune compromised states, and various hypoperfusion states such as prolonged septic shock. Moreover, the dermal Shwartzman reaction may yet prove useful in investigations into the molecular explanation for human cases of *purpura fulminans* from unusual environmental bacterial infections from such pathogens such as *Aeromonas*, *Vibrio*, or *Capnocytophaga* spp. (see Table 3).[91–104]

Like most disciplines in scientific inquiry, attempts at understanding the basic nature of human host response to infection have had its share of miscues, false leads, failed hypotheses, and tragic mistakes.[105,106] The pace of discovery is quickening as technologic advances in molecular biology keep churning out new challenges to established ideas and long-standing hypotheses. No matter how compelling the experimental evidence, mistakes will be made when trying to bring new ideas from the bench to the bedside. We need to realize and accept the fact that new research studies involving human subjects and vulnerable patients will inevitably carry some intrinsic risk. Fortunately, the mistakes, ethical blunders, and sometimes egregious human errors made while conducting clinical trials in the 19th and 20th century will serve as an ethical guide for biomedical research in the present and future. We owe it to our patients to live up to the high standards placed upon us and expected from us.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

References

1. Robbins SL and Angell M. Disorders of Immunity. In: *Basic Pathology*. Philadelphia: Saunders, 1971, pp. 138–167
2. Shwartzman G. Studies on *Bacillus typhosus* toxic substances. 1. Phenomenon of local skin reactivity to *B. typhosus* culture filtrate. *J Exp Med* 1928; 48: 247–268.
3. Centanni E. Untersuchungen über das Infections Fieber—das Fiebergift der Bakterien. *Dtsch Med Wochenschr* 1894; 20: 148–150.
4. Centanni E. Immunitatserscheinungen im Experimentellen Fieber mit Besonderer Berucksichtigung des Pyrogenen Stoffs aus Typhusbakterien. *Klin Wochenschr* 1942; 21: 664–669.
5. Heyman A. The treatment of neurosyphilis by continuous infusion of typhoid vaccine. *Vener Dis Inform* 1945; 26: 51–57.
6. Antopol W. The production of the Shwartzman phenomenon by means of bacterial extracts. *J Infect Dis* 1937; 61(3): 331–333.
7. Bell WR, Miller RE and Levin J. Inhibition of the generalized Shwartzman reaction by hypofibrinogenemia. *Blood* 1972; 40: 697–708.
8. Sanarelli G. Le cholera experimental. *Ann Inst Pasteur* 1924; 37: 11–80.
9. Arthus NM. Injections répétées de serum du cheval chez le lapin. *C R Seances Soc Biol Fil* 1903; 55: 817–820.
10. Biswas SK and Lopez-Collazo E. Endotoxin tolerance: new mechanisms, molecules and clinical significance. *Trend Immunol* 2009; 30: 475–487.
11. Foster SL, Hargreaves DC and Medzhitov R. Gene-specific control of inflammation by TLR-induced chromatin modifications. *Nature* 2007; 447: 972–978.
12. Novakovic B, Habibi E, Wang S-Y, et al. β-Glucan reverses the epigenetic state of LPS-induced immunological tolerance. *Cell* 2016; 167: 1354–1368.
13. De Lima TM, Sampaio SC, Petroni R, et al. Phagocytic activity of LPS tolerant macrophages. *Mol Immunol* 2014; 60: 8–13.
14. Coley WB. The treatment of malignant tumors by repeated inoculations of erysipelas: with report of ten original cases. *Am J Med Sci* 1893; 105(5): 488–511.
15. Movat HZ, Burrowes CE, Cybulsky MI, et al. Acute inflammation and a Shwartzman-like reaction induced by interleukin-1 and tumor necrosis factor. *Am J Pathol* 1987; 129(3): 463–476.
16. Tsung K and Norton JA. Lessons from Coley's toxin. *Surg Oncol* 2006; 15: 25–28.
17. Shwartzman G. Hemorrhagic necrosis and regression sarcoma 180. *Science* 1935; 82: 201–202.
18. Ogata O. Inhibition of the Shwartzman phenomenon. *Tokyo Igakkwai Zasshi* 1932; 46: 2390–2399.
19. Thomas L and Good RA. Studies on the generalized Shwartzman reaction: I. General observations concerning the phenomenon. *J Exp Med* 1952; 96(6): 605–624.
20. Thomas L and Good RA. Studies on the generalized Shwartzman reaction. II. The production of bilateral cortical necrosis of the kidneys by a single injection of bacterial toxin in rabbits previously treated with thorotrast or trypan blue. *J Exp Med* 1952; 96(6): 625–641.
21. Thomas L and Good RA. The effect of cortisone on the Shwartzman reaction; production of lesions resembling the dermal and generalized Shwartzman reaction by a single injection of bacterial toxin in cortisone-treated rabbits. *J Exp Med* 1952; 95(4): 409–428.

22. Stetson CA Jr. Studies on the mechanism of the Shwartzman phenomenon; certain factors involved in the production of local hemorrhagic necrosis. *J Exp Med* 1951; 93(5): 489–504.
23. Thomas L and Stetson CA Studies on the mechanism of the Shwartzman phenomenon. *J Exp Med* 1949 89: 461–478.
24. Schade FU, Flach R, Flohe S, et al. Endotoxin tolerance. In: Brade H, Opal SM, Vogel SN, et al. (eds) *Endotoxin in health and disease*. New York: Dekker, 1999, pp. 751–768.
25. Fehniger TA, Yu H, Cooper MA, et al. Cutting edge: IL-15 co-stimulates the generalized Shwartzman reaction and innate immune IFN-gamma production in vivo. *J Immunol* 2000; 164: 1643–1647.
26. Ziegler EJ, Douglas H and Braude AI. Human antiserum for the prevention of the local Shwartzman reaction and death from bacterial lipopolysaccharides. *J Clin Invest* 1973; 52: 3236–3238.
27. Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. *N Engl J Med* 2015; 373: 123−135
28. Dobrina A, Schwartz BR, Carlos TM, et al. CD11/CDI8-independent neutrophil adherence to inducible endothelial-leukocyte adhesion molecules (ELAM) in vitro. *Immunology* 1989; 67: 502–508
29. Dustin ML and Springer T. Lymphocytes function-associated antigen 1 (LFA-1) interaction with intercellular adhesion molecule (ICAM-1) is one of at least three mechanisms for lymphocyte adhesion to cultured endothelial cells. *J Cell Biol* 1988; 107: 321–331
30. Dustin ML, Rothlein R, Bhan AH, et al. Induction by IL- I and interferon-alpha tissue distribution, biochemistry and function of a natural adherence molecule (ICAM-1). *J lmmunol* 1986; 137: 245–254.
31. Mashimo J-I, Tanaka C, Arata S, et al. Structural heterogeneity regarding local Shwartzman activity of lipid A. *Microbiol Immunol* 1988; 32(7): 653–666.
32. Kanagasaki S, Tanamoto K-I, Yasuda T, et al. Structure-activity relationship of lipid A: Comparison of biologic activities of natural and synthetic lipid A's with differential fatty acid composition. *J Biochem* 1986; 99: 1203–1210.
33. Park BS, Song DH, Kim HM, et al. The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex. *Nature* 2009; 458: 1191–1196.
34. de Kruis P. *Microbe hunters*. New York: Harcourt, Brace and Company, 1926, pp 1–358.
35. Opal SM. A brief history of microbiology and immunology. In: Artenstein AW (ed) *Vaccines: A Biography*. New York: Springer, 2010, pp. 31–56.
36. Lowy I. Yellow Fever in Rio de Janeiro and the Pasteur Institute mission (1901–1905): The transfer of science to the periphery. *Med History* 1990; 34: 144–163.
37. Calmette A. Les missions scientifiques de l'Institut Pasteur et l'expression colonial de la France. *Rev Scient* 1912; 89: 129–132.
38. Crosby MC. *The American plague: The untold story of Yellow Fever, the epidemic that shaped our history*. New York: Berkley Books, 2006.
39. Sutton JH and Harrison JB. The micro-organism of Yellow Fever. *Lancet* 1885; i: 405–406.
40. Sanarelli G. Etiolgie et pathogenese de la fievre jaune. *Ann Inst Pasteur* 1897; 11(6): 433–512.
41. Sanarelli G. Das myxomatogene virus. *Zentralblatt fur Bakteriologie und Parasitenkunde* 1898; 23: 865–879.
42. Kerr P and McFadden G. Immune responses to myxoma viruses. *Viral Immunol* 2002; 15: 229–246.
43. Spiesschaert B, McFadden G, Hermans K, et al. The current status and future directions of myxoma virus, a master in immune evasion. *Vet Res* 2011; 42(1): 76–87.
44. Burnet FM. Myxomatosis as a method of biological control against the Australian rabbit. *Am J Public Health Nations Health* 1952; 42: 1522–1526.
45. Artenstein A, Opal JM, Opal SM, et al. The role of the United States military in vaccine development: a historical review. *Military Med* 2005; 170(4): 3–10.
46. Guerena-Burgueno F. The centennial of the Yellow Fever Commission and the use of informed consent in medical research. *Salud Publica Mex* 2002; 44: 140–144.
47. Reed W and Carroll J. *Bacillus icteroides* and *Bacillus cholerae suis*: A preliminary note. *Med News* 1899; 74: 513.
48. Reed W, Agramonte A and Lazear J. The etiology of Yellow Fever—a preliminary note. *Philadelphia Med* 1900; 148(6): 101.
49. Sanarelli G. Premiere experiences sur l'emploi du serum curative et preventif de la fievre jaune. *Ann Inst Pasteur* 1898; 12: 348–360.
50. World Health Organization: Declaration of Helsinki for Medical Ethics. http://www.who.int/bulletin/volumes/86/8/08-050955/en/ (1964/2000, accessed 16 October 2018).
51. Dhai A. The research ethics evolution: From Nuremberg to Helsinki. *S Afr Med J* 2014; 104(3): 178–180.
52. Jenner E. *An inquiry into the causes and effects of the variolae vaccine: a disease discovered in some western counties in England, particularly Gloucestershire, and known by the name cowpox*. Birmingham, Alabama: The Classics of Medicine Library Division of Gryphon Editions, 1798.
53. Stein R. US apologizes for newly revealed syphilis experiments done in Guatemala. Washington Post, 1 October 2010.
54. Lederer SE. *Subjected to science: Human experimentation in America before the Second World War*. Baltimore, MD: Johns Hopkins University Press, 1995, pp. 1–192.
55. Sanarelli G. De la pathogenie du cholera; Voies de penetration et de sortie des vibrions choleriques dans 1-organisme animal. *Ann Inst Pasteur* 1923; 34: 364–376.
56. McKay DG, Gitlin D and Craig JM. Immunochemical demonstration of fibrin in the generalized Shwartzman reaction. *Arch Path* (Chicago) 1959; 69: 270–273.
57. Scott GBD. Observations on fibrin thrombi produced by endotoxins: an experimental study. *Br J Exp Pathol* 1962; 43: 93–101.
58. Margaretten W, Zunker HO and McKay DG. Production of the generalized Shwartzman reaction in pregnant rats by intravenous infusion of thrombin. *Lab Invest* 1964; 13: 552–559.

59. Levi M and Opal SM. Coagulation abnormalities in critically ill patients. *Crit Care* 2006; 10(4): 222.
60. Levi M, Schultz M and van der Poll T. Sepsis and thrombosis. *Semin Thromb Hemost* 2013 Jul; 39(5): 559–566.
61. Lyons PG, Micek ST, Hampton N, et al . Sepsis-associated coagulopathy severity predicts hospital mortality. *Crit Care Med* 2018; 46: 736–742.
62. Deppermann C and Kubes C. Start a fire, kill the bug: The role of platelets in inflammation and infection. *Innate Immun* 2018; 24(6): 335–348.
63. Deutschman CS and Tracey KJ. Sepsis: Dogma and new perspectives. *Immunity* 2014; 40: 463–475.
64. Hotchkiss RS, Moldawer LL, Opal SM, et al. Sepsis and septic shock. *Nat Rev Dis Primers* 2016; 2: 1–21.
65. Restagno D, Venet F, Paquet C, et al. Mice survival and plasmatic cytokine secretion in a "Two Hit" model of sepsis depend on Intratracheal *Pseudomonas aeruginosa* bacterial load. *PLoS One* 2016; 11(8): e0162109.
66. Eissner B, Matz K, Smorodchenko A, et al. Chronic porcine Two-Hit model with hemorrhagic shock and *Pseudomonas aeruginosa* sepsis. *Eur Surg Res* 2002; 34: 61–67
67. Gisely Amaranteeixeira da Cunha M, Bastos de Albuquerque D, Chaves D, et al. Role of inflammatory caspases in a murine two-hit model of sepsis: analysis of immunosuppression and cognitive impairment. *Critical Care* 2013; 17(Suppl 4): 105.
68. Shwartzman G. A new phenomenon of local skin reactivity to *B. typhosus* culture filtrate. *Proc Soc Exp Biol Med* 1928; 25: 560–561
69. Shiga J and Mori W. A study on pulmonary hemorrhage experimentally produced by the Shwartzman mechanism in rabbits. *Acta Pathol Jpn* 1985; 35: 849–861.
70. Mori W. The Shwartzman reaction: a review including clinical manifestations and proposal for a univisceral or single organ third type. *Histopathology* 1981; 5: 113–126.
71. Hjort PF and Rapport SI. The Shwartzman Reaction: Pathogenetic mechanisms and clinical manifestations. *Annu Rev Med* 1965; 16: 135–168.
72. Thal AP and Brackney E. Acute hemorrhagic pancreatic necrosis produced by local Schwartzman reaction. *JAMA* 1954; 155: 569–576.
73. Kondo M, Takemura S, Yokoe N, et al. Gastrointestinal hemorrhage due to increased blood coagulation. I. Experimental disseminated intravascular coagulation by bacterial endotoxin. *Jpn J Gastroenterol* 1976; 73: 1373–1380.
74. Mori W. The Shwartzman reaction: a review including clinical manifestations and proposal for a univisceral or single organ third type. *Histopathology* 1981; 5: 113–126.
75. Penington AJ, Craft RO, Mitchell G, et al. Local Shwartzman reaction in the rat induced by endotoxin and ischemia: potential model for skin necrosis in meningococcemia. *Pediatr Res* 2006; 60(2): 158–160.
76. Iskander KN, Osuchowski MF, Stearns-Kurosawa DJ, et al. Sepsis: multiple abnormalities, heterogeneous responses, and evolving understanding. *Physiol Rev* 2013 Jul; 93(3): 1247–1288.
77. Delabranche X, Stiel L, Severac F, et al. Evidence for netosis in septic shock-induced disseminated intravascular coagulation. *Shock* 2017: 47(3): 313–317.
78. Lee LJ. Reticuloendothelial clearance of circulating fibrin in the pathogenesis of the generalized Shwartzman reaction. *J Exp Med* 1962; 115: 1065,
79. Levi M and van der Poll T. Coagulation and sepsis. *Thromb Res* 2017; 149: 38–44.
80. Shwartzman C, Klemperer P and Gerber IE. Phenomenon of local tissue reactivity to bacterial filtrates: Role of altered vascular response in certain human diseases. *JAMA* 1936; 107: 1946.
81. Shwartzman G. *Phenomenon of local tissue reactivity*. New York: Paul B. Hoeber, 1937.
82. Davis CE and Arnold K. Role of meningococcal endotoxin in meningococcal purpura. *J Exp Med* 1974; 140: 159–171.
83. Francis RB. Acquired purpura fulminans. *Semin Thromb Hemost* 1990; 16: 310–325.
84. Lécuyer H, Virion Z, Barnier J-P, et al. An ADAM-10 dependent EPCR shedding links meningococcal interaction with endothelial cells to purpura fulminans. *PLOS Pathogens* 2018; 14(4): e1006981.
85. Faust SN, Levin M, Harrison OB, et al. Dysfunction of endothelial protein C activation in severe meningococcal sepsis. *N Engl J Med* 2001; 345: 408–416.
86. Manco-Johnson MJ, Abshire TC, Marlar RA. Severe neonatal protein C deficiency: prevalence and thrombotic risk. *J Pediatr* 1991; 119(5): 793–798.
87. Lyons PG, Micek ST, Hampton N, et al. Sepsis-associated coagulopathy severity predicts hospital mortality. *Crit Care Med* 2018; 46(5): 736–742.
88. Tanaka K, Jun T, Matsumoto H, et al. Anticoagulation therapy using rh-thrombomodulin or anti-thrombin III agents is associated with a reduction in in-hospital mortality in septic disseminated intravascular coagulation: A nation-wide registry study. *Shock* 2018. Epub ahead of print July 30 2018. DOI: 10.1097/SHZ.0000000000001230
89. Yamakawa K, Aihara M, Ogura H, et al. Recombinant human thrombomodulin in severe sepsis: A systematic review and meta-analysis. *J Thromb Haemost* 2015; 13(4): 508–519.
90. Cohen J, Vincent J-L, Adhikari FR, et al. Sepsis: a roadmap for future research. *Lancet Infect Dis* 2015; 15: 581–614
91. Tyring SK and Lee PC. Hemorrhagic bullae associated with *Vibrio vulnificus* septicemia. *Arch Dermatol* 1986; 122: 818–820.
92. Horseman MA and Surani S. A comprehensive review of *Vibrio vulnificus*: an important cause of severe sepsis and skin and soft-tissue infection. *International J Infect Dis* 2011; 15(3): e157–e166.
93. Choi H-J, Lee D-K, Lee M-W, et al. *Vibrio vulnificus* septicemia presenting as purpura fulminans. *J Dermatol* 2014; 32(1): 1–6.
94. Hor L-I and Chen C-L. Cytotoxins of *Vibrio vulnificus*: Functions and roles in pathogenesis. *BioMed* 2013; 3(1):19–26.
95. Liu B-M, Changchien C-S, Chen C-H, et al. Fatal *Aeromonas hydrophila* infection after endoscopic

injection sclerotherapy for gastric variceal bleeding. *Chang Gung Med J* 2004; 27: 766–769.
96. Thisyakorn U and Ningsanond V. Purpura fulminans produced by *Aeromonas hydrophila:* A case report. *Southeastern Asian J Trop Med Public Health* 1986:16(4): 532–533.
97. Kravitz GR, Dries DJ, Peterson ML, et al. Purpura fulminans due to *Staphylococcus aureus*. *Clin Infect Dis* 2005; 40: 941–947.
98. Ward KM, Celebi JT, Gmyrek R, et al. Acute infectious purpura fulminans associated with asplenism or hyposplenism. *J Am Acad Dermatol* 2002; 47(4): 493–496.
99. Asakura T, Higuchi A and Mori N. *Streptococcus pneumoniae*-induced purpura fulminans. *QJM International J Med* 2016; 109(7): 455–500.
100. Christiansen CB, Berg RMG, Plovsing RR, et al. Two cases of infectious purpura fulminans and septic shock caused by *Capnocytophaga canimorsus* transmitted from dogs. *Scand J Infect Dis* 2012; 44: 635–639.
101. Grimaldi D, Bonacorsi S, Roussel H, et al. Unusual "flesh-eating" strain of *Escherichia coli*. *J Clin Microbiol* 2010; 48(10): 3794–3796.
102. Amara M, Bonacorsi S, Bedel J, et al. Group A *Escherichia coli*-related purpura fulminans: An unusual manifestation due to an unusual strain. *J Clin Microbiol* 2014; 52(12): 4404–4406.
103. Opal SM, Artenstein A, Cristofaro PA, et al. Inter-alpha inhibitor proteins are endogenous furin inhibitors and provide protection against experimental anthrax intoxication. *Infect Immun* 2005; 73(8): 5101–5105.
104. Cohen R, Babushkin F, Shapiro M, et al. Two cases of Israeli Spotted Fever with purpura fulminans, Sharon District, Israel. *Emerg Infect Dis* 2018; 24(5): 835–840.
105. Suntharalingam G, Perry MR, Ward S, et al. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN 412. *N Engl J Med* 2006; 355: 2591–2594.
106. Raper SE, Chirmule N, Lee SL, et al. Fatal systemic ornithine transcarbamylase gene transfer. *Mol Gene Metabol* 2003; 80(1–2): 148–158.