Toxic Shock Syndrome

and the Other

Staphylococcal Toxicoses

By

Hans-Christian Mittag, Düsseldorf

With 20 Figures and 24 Tables

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Preface

This book is based on my thesis "Staphylokokkentoxikosen unter besonderer Berücksichtigung des Toxic Shock Syndrome" (1000), which was written in winter 1984/85 and printed in German in 1986. Given the high incidence of toxic shock syndrome in the USA, and the great attention it attracted, it seemed appropriate to make this overview available to the interested American reader in form of an English edition.

Because many new facts about TSS and in related areas of investigation were discovered since 1984, the contents were completely revised and greatly expanded. It was my intention to facilitate orientation and increase readability, while at the same time trying to give a complete inventory of the current knowledge about TSS.

I wish to thank Professor Dr. L. GRÜN for the basic idea, founded on decades of experience with staphylococcal research. I am indebted to my mother, Dr. J. ESSER MITTAG, for constructive comments and invaluable support in the preparation of the manuscript. I want to thank everyone at the Schattauer Verlag, who helped to transform the manuscript into a fine book. And finally I want to apologize to my wife Marion and my children Kira, Nikolas and little Timo for the recurrent deprivation while their husband and father pursued his scientific objectives.

Meerbusch, July 1987 H.-C. MITTAG

List of Abbreviations

ADP	Adenosine diphosphate
AGR	Accessory gene regulator
AMP	Adenosine monophosphate
ARDS	Adult respiratory distress syndrome
CDC	Centers for Disease Control
CGRP	Calcitonin gene-related peptides
CMC	Carboxymethylcellulose
CNS	Central nervous system
CPK	Creatine phosphokinase
D	Dalton
DB	Dutch-belted (rabbits)
DNA	Desoxyribonucleic acid
ED_{50}	Median effective dose
ELISA	Enzyme-linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
ET (A, B)	Exfoliatin (A, B)
GOT	Glutamic-oxalacetic transaminase
GPT	Glutamic-pyruvic transaminase
HLA	Histocompatibility leukocytic antigen
HTLV III	Human T-cell leukemic virus III
ICD	International Classifications of Diseases
IL	Interleukin
IS	Inhibiting substance
IUD	Intrauterine device
kD	Kilo Dalton
LAF	Lymphocyte activating factor
LD_{50}	Median lethal dose

LDH	Lactic dehydrogenase
LPS	Lipopolysaccharide = Endotoxin
MAF	Macrophage activating factor
mRNA	messenger RNA
NADH	Nicotinamide-dinucleotide (reduced)
NADP	Nicotinamide-dinucleotide phosphate
NZW	New-Zealand white (rabbits)
PAP	Pulmonary arterial pressure
PE (A–C)	Pyrogenic exotoxin (A–C)
PEEP	Positive endexpiratory pressure
PMN	Polymorphonuclear neutrophils
PT	Prothrombin time
PTH	Parathormone
PTT	Partial thromboplastin time
RES	Reticuloendothelial system
RME	Receptor-mediated endocytosis
RNA	Ribonucleic acid
SE (A–F)	Staphylococcal enterotoxin (A–F)
SP (A–C)	Pyrogenic streptococcal exotoxin
SPF	Streptococcal proliferative factor
SPRIA	Solid phase radioimmunoassay
SSSS	Staphylococcal skalded skin syndrome
TEN	Toxic epidermal necrolysis
TSA	Toxic shock-associated antigen
TSS	Toxic shock syndrome
TSST-1	Toxic shock syndrome toxin 1
TST	Toxic shock toxin
WBC	White blood cells

Contents

Introduction

The term toxic shock syndrome (TSS) was coined by Dr. JAMES TODD who had observed a clinical picture characterized by high fever, generalized erythroderma, hypotensive shock and involvement of several organ systems in seven children – 4 girls and 3 boys. He found staphylococci of phage group I in all these patients. At the end of 1978, TODD published his observations (870).

The symptom complex was not new per se. It had already been variously described, usually diagnosed as staphylococcal scarlet fever or Kawasaki syndrome, (see Chap. 3.15) but had never received the attention that it would soon attract under the new name.

The public furor about TSS began in the spring of 1980 when a number of cases of the disease in young women provided abundant material for attention-grabbing headlines in American newspapers: "Young women suddenly struck by new toxic shock syndrome". Agitation increased that summer when a number of epidemiological statistics revealed that users of tampons were especially at risk: "Alarm in the USA: toxic death due to tampons". In the summer of 1980, half a year after the first publications, results of polls showed that practically every American woman had heard of toxic shock syndrome.

Seven years have passed in the meantime, the public has settled down, and science has made a major accomplishment, just as with the two other "new" infectious diseases of the last ten years, legionnaire's disease and AIDS. A large body of facts were collected with unheard of speed, despite the occasional burden of public pressure. Staphylococcus research was inspired, especially work with staphylococcal toxins, and previously neglected research fields such as vaginal physiology and microbiology became the subject of greater interest. Nevertheless, it is still impossible today to put together a complete picture from all the collected pieces of the mosaic. Many pieces are still missing and central questions remain unanswered.

It was surprising for many that "only" Staphylococcus aureus could be isolated in the patients. Staphylococci are known as pathogens in trivial skin affections, banal diarrheas, and osteomyelitis, but they were not thought to be capable of causing an illness of such severity.

Staphylococci have already been a cause for concern many times in medical history. From the deaths due to postoperative staphylococcal septicemia prior to the introduction of asepsis to staphylococcal pneumonias during the influenza epidemic of 1918 to the worldwide pediatric, gynecological, and surgical infections

caused by phage type 80/81 in the 1950s (656, 961) and recently the spread of methicillin-resistant strains (859), staphylococci have repeatedly proven their adaptability to altered environmental conditions. Development of multiple resistances to antibiotics has led many a physician to the limits of therapeutic options.

Highly toxic staphylococcal infections have also been described frequently since Sir ALEXANDER OGSTON coined the name "Staphylococcus" in the year 1881.

Against this background, the emergence of a syndrome such as TSS is less surprising. It shows what a versatile opponent we have to face.

Staphylococci produce a variety of extracellular substances, several of which are capable of triggering toxic diseases. The purpose of this study is to permit comparisons by means of a synopsis of the various pathological conditions caused by staphylococcal toxins and to find a place for toxic shock syndrome in the broad spectrum of staphylococcal diseases, after it became somewhat unmangeable due to the great publicity.

1. Staphylococcal Toxins

The presence of toxic substances in culture filtrates of staphylococci was described repeatedly in the last century (121, 187, 517, 896). In the tragedy of Bundaberg in 1928 when 11 children died after being injected with a vaccine infected with a toxin-producing strain of staphylococcus (465; see also Chap. 2.3), Staphylococcus aureus demonstrated its ability to produce potent toxins in a tragic manner and prompted extensive investigation of these substances. However, the difficulty of isolating the toxins in pure form in the past has led to confusion in nomenclature and contradictory descriptions of biological properties. Even after improving the isolation methods, many questions still remain regarding the role of these substances in pathogen-host interactions in staphylococcal infections.

According to BONVENTRE, a **toxin** is a high molecular weight protein with antigenic action which causes a disturbance of the normal physiological processes in sensitive individuals (102).

However, it is hardly possible to draw a clear distinction between staphylococcal toxins and other extracellular staphylococcal products, because the manner of their involvement in various disease processes is largely unexplained. Various substances do not cause cell damage when alone, but when combined with others, they develop toxic effects (e. g., nucleases). With some substances toxic effects can be produced in vitro, but little is known about their effect in vivo (e.g., gamma toxin). And even for other substances recognized as toxins, synergisms with other staphylococcal products have occasionally been postulated as essential for the effect (e. g., exfoliatin, possibly enterotoxins).

Table 1 summarizes the most important staphylococcal products and their effects in key words. At the present, a total of 20–30 extracellular proteins are known (599) and others are thought to exist (907).

Exfoliatin and *enterotoxins* can be assigned to relatively clearly defined clinical pictures. Therefore, they are treated separately in the discussion of staphylococcal toxicoses in Chap. 2.

The attention drawn to *pyrogenic toxins* in combination with toxic shock syndrome has prompted a separate consideration in Chap. 3.4.4.

The other staphylococcal products will be discussed briefly below with regard to their most important properties.

Table 1. Products of staphylococci.

Extracellular	
Alpha toxin	Hemolysis, lethality, cytotoxicity
Beta toxin	Sphingomyelinase C, hemolysis, cytotoxicity
Gamma toxin	Hemolysis, cytotoxicity
Delta toxin	Surfactant-like activity, hemolysis, cytotoxicity
Epidermolytic toxins	Epidermolysis, causative agents of SSSS (see Chap. 2.1)
Pyrogenic toxins	Disputable as separate entities (TSST-1 might belong here)
Mitogenic factors	Mitogenic for lymphocytes; maybe identical with pyrogenic toxins (3
Leukocidin	Leukotoxicity
Coagulase	Complexing with prothrombin leads to clotting of blood
Enterotoxins	Effects on various organ systems (see Chap. 2.2)
Bacteriocins, micrococcins	Bactericidal and bacteriostatic activity in vitro
Lysozyme	Hydrolysis of peptidoglycan
Nuclease	Hydrolysis of RNA and DNA
Hyaluronidase	Hydrolysis of hyaluronic acid
Decomplementation antigen	Consumption of complement factors (89)
Succinat-oxidase factor	Inhibition of mitochondrial metabolism (320)
Cellular	
Peptidoglycan	Pyrogenicity, adjuvancity, immunomodulation
Capsular substance	Antiphagocytotic, protection against antibiotics, immunomodulation (671)
"Clumping factor"	Paracoagulation of fibrinogen
Protein A	Reaction with Fc fragment of immunoglobulins

(Complemented after 429.)

1.0.1. Alpha Toxin

Alpha toxin is one of the best researched staphylococcal products. It is a heterogeneous protein with a molecular weight of 26 to 39 kD and an isoelectric point of 8.5. In a highly purified state, it is relatively unstable. It is formed by about 85% of clinical isolates of Staphylococcus aureus (599).

It is chromosomally coded (445), possibly on a transposon. There are indications that its expression is controlled by a regulator gene (301). The base sequence of the alpha toxin gene of the "Wood 46" strain has been elucidated, and the respective

protein has a molecular weight of 33 kD. The gene includes a "signal peptide" that contains 26 amino acids (331).

Alpha toxin has a membrane-damaging effect on a wide variety of cells. Its effect is based on the formation of *ring hexamers* (inside diameter 2–3 nm, outside diameter 10 nm, height 5–6 nm) in contact with biological membranes. These ring hexamers become embedded in the biomembrane and thus permit the passage of smaller molecules up to a molecular weight of about 4–6 kD (86, 310, 843). Increased influx of calcium ions into the cell then causes other pathophysiological effects by activating the arachidonic acid cascade and releasing prostaglandins (785). The intoxication is independent of metabolic energy, active receptor clustering on the cell surface and endocytosis of the toxin (96).

The effect of alpha toxin is manifested in both warm-blooded and cold-blooded species, but it differs greatly in extent depending on the form of administration and species (429). Separate regions of the alpha-toxin molecule are responsible for binding and biological activities (97). The existence of a receptor protein is not yet totally proven (552). Certain tissues seem to be especially sensitive, possibly due to their myelin content (355). *Rabbit erythrocytes* are most sensitive to the hemolytic action (301), at physiologic pH. At low pH levels other membranes become susceptible too (995).

Because of the instability of alpha toxin, the ease with which it is inactivated in purification and workup processes (445) and, especially in the past, the risk of contamination (e. g., with delta toxin), there are contradictory research results with regard to its effects on the entire body. It will be necessary to reexamine many experiments.

The following effects are attributed to alpha toxin:

Lethal effect: So far no warm-blooded animal species has been found that is resistant to the lethal effect of alpha toxin preparations (908; LD_{50}: mouse = 1 µg, rabbit = 4 µg; 301). Fatal staphylococcal infections in humans have also been attributed to this toxin (435, 642). The lethal effect is presumably due to a direct action on the central nervous system (355). Rabbits receiving the minimum lethal dose died after a few days, usually developing a flaccid paresis of the rear legs first. Pathological findings included mainly kidney necroses. Shortness of breath, muscular spasms, intravascular hemolysis and death after a few minutes occur at higher doses (728).

Central nervous system: Alpha toxin presumably acts mainly on thalamic structures and leads to an interruption in the connection between mesencephalon and diencephalon presumably by damaging the myelin sheaths of the nerve fibers. Myelin is especially sensitive to alpha toxin (354, 355, 908).

Circulation: Alpha toxin increases vascular permeability (e. g., via histamine and serotonin from mast cells and platelets). The secretion of catecholamines from the adrenals is regarded as responsible for hypertensive effects (428, 908).

Lung: In perfusion (0.04 µg/ml) of isolated rabbit lung, alpha toxin causes the perfusion pressure to increase 4 to 16 mmHg. The reaction is triggered by activation of the vascular arachidonic acid cascade independently of mediators from plasma and circulating cells (785). The arachidonic acid cascade is also triggered in isolated pulmonary arterial endothelial cells (786).

Heart: ECG changes seem to be a result of the CNS involvement (908). Perfusion of isolated rabbit hearts with 2µg/ml alpha-toxin leads to increased coronary resistance, diastolic wall thickness and enddiastolic pressure (144).

Kidney: Bilateral cortical necrosis has been observed, presumably due to vasoconstrictory ischemia, different from the generalized Schwartzmann reaction. Plasminogen activation might also play a role (428, 908).

Smooth musculature: Alpha toxin acts directly on smooth muscle cells and leads to spastic contraction (908).

Skin: Administered intradermally, alpha toxin leads to local dermonecrosis – whether due to a direct toxic effect or vasospasm is undetermined (908).

Immune system: Alpha toxin has a nonspecific stimulating effect on lymphocytes. This is independent of the cell damaging effect (678).

Granulocytes: In small doses the phagocytosis of Staphylococcus aureus is increased, but in large doses it is inhibited (319).

Teratogenic effect: Alpha toxin has led to abortions in rabbits (428, 908).

Distribution in the body (rabbits): The toxin reaches practically every organ. The largest concentrations are found in kidney and lung, relatively high concentrations are also found in the brain (728).

1.0.2. Beta Toxin

Beta toxin is a heat-labile protein with a molecular weight of 11 kD to 38 kD and an isoelectric point of 9.4 (429). Highly purified, it decomposes at −20 °C within a few days (301).