The Role of Streptococcal and Staphylococcal Exotoxins and Proteases in Human Necrotizing Soft Tissue Infections

Patience Shumba, Srikanth Mairpady Shambat and Nikolai Siemens

1 Center for Functional Genomics of Microbes, Department of Molecular Genetics and Infection Biology, University of Greifswald, D-17489 Greifswald, Germany; patience.shumba@uni-greifswald.de
2 Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, CH-8091 Zurich, Switzerland; Srikanth.MairpadyShambat@usz.ch
* Correspondence: nikolai.siemens@uni-greifswald.de; Tel.: +49-3834-420-5711

Received: 20 May 2019; Accepted: 10 June 2019; Published: 11 June 2019

Abstract: Necrotizing soft tissue infections (NSTIs) are critical clinical conditions characterized by extensive necrosis of any layer of the soft tissue and systemic toxicity. Group A streptococci (GAS) and Staphylococcus aureus are two major pathogens associated with monomicrobial NSTIs. In the tissue environment, both Gram-positive bacteria secrete a variety of molecules, including pore-forming exotoxins, superantigens, and proteases with cytolytic and immunomodulatory functions. The present review summarizes the current knowledge about streptococcal and staphylococcal toxins in NSTIs with a special focus on their contribution to disease progression, tissue pathology, and immune evasion strategies.

Keywords: Streptococcus pyogenes; group A streptococcus; Staphylococcus aureus; skin infections; necrotizing soft tissue infections; pore-forming toxins; superantigens; immunomodulatory proteases; immune responses

Key Contribution: Group A streptococcal and Staphylococcus aureus toxins manipulate host physiological and immunological responses to promote disease severity and progression.

1. Introduction

Necrotizing soft tissue infections (NSTIs) are rare and represent a more severe rapidly progressing form of soft tissue infections that account for significant morbidity and mortality [1]. NSTIs can be classified according to the invading organisms (types I–III), and less commonly, the depth of invasion, or anatomic location (trunk, extremity, perineum) [1–4]. Type I NSTIs, also referred to as synergistic NSTIs, affect around 70%–80% of patients seen in practice [1,3]. They are of a polymicrobial nature, frequently involving a mixture of aerobic and anaerobic bacteria [5] and affect elderly and/or patients with multiple underlying conditions, including diabetes mellitus, obesity, vascular diseases, renal insufficiency, and immunosuppression [6]. Type II NSTIs, causing around 20%–30% of cases, are of a monomicrobial nature mostly due to Gram-positive organisms. Among these, Streptococcus pyogenes (group A streptococcus [GAS]) is the most common pathogen [7–10]. Although S. aureus has not been described as a monomicrobial cause of NSTIs in clinical settings until 2005, the number of methicillin-resistant S. aureus (MRSA) NSTIs is constantly increasing leading to the second major species responsible for type II NSTIs [11]. Type II NSTIs affect mostly young individuals without underlying conditions with a recent history of trauma to an extremity or intravenous drug abuse [4]. Type III infections are confined to warm coastal areas and are caused mainly by Gram-negative Vibrio...
species [1,12]. This review article focuses solely on type II NSTIs caused by GAS and S. aureus and the role of respective exotoxins and secreted proteases contributing to the severity of infection.

2. Pathophysiology of Type II NSTIs

GAS and S. aureus are Gram-positive cocci, which share many features, including clinical aspects and pathogenic mechanisms. Both secrete virulence factors with pore-forming and/or immunomodulatory properties (Figure 1). However, they also have unique features. S. aureus is a major cause of community- and hospital-acquired infections ranging from mild superficial skin and throat infections to invasive infections such as toxic shock syndrome (TSS) and NSTIs [13]. A great public health concern is the increasing prevalence of MRSA, specifically the rise in community-acquired (CA) S. aureus [13–15]. Specifically CA-MRSA clones are associated with highly aggressive infections, including NSTIs, in otherwise healthy individuals [11]. GAS with an estimate of 500,000 deaths annually is rated as number nine on the list of global killer pathogens [16]. GAS can cause a variety of diseases in immunocompetent individuals similar to those listed for S. aureus [16].

![Figure 1. Streptococcal and staphylococcal secreted virulence factors with pore-forming and/or immunomodulatory properties. (a) Group A streptococcal (GAS) secreted factors: Streptolysins S and O (SLS, SLO), streptococcal pyrogenic exotoxin B (SpeB), superantigens (SAgs), C5a peptidase (ScpA), Immunoglobulin degrading enzyme of streptococci (IdeS), SpyCEP, SpyA, Streptokinase (Ska), and NADase. (b) Staphylococcal secreted factors: Leukocidins, α-toxin, phenol-soluble modulins (PSMs), superantigens (SAgs), staphopain A (ScpA), Staphopain B (SspB), Aureolysin (Aur), V8 protease, exfoliative toxins (ETs), epidermin leader processing protease (EpiP), serine protease-like proteins (Spls), and staphylokinase (SAK).](image-url)
necrosis of the deeper tissue that spreads to upper tissue layers. In contrast to NSTIs with a defined portal of entry, the bullae and ecchymoses develop later [4].

3. Superantigens and Toxic Shock Syndrome

Invasive GAS infections are often complicated by streptococcal toxic shock syndrome (STSS) [19]. According to Sepsis-3 consensus, sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection. Toxic shock is a subset of sepsis in which particularly profound circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone [20]. Approximately 50% of GAS NSTI cases are associated with STSS [21,22], which significantly increases the mortality of GAS NSTIs [21,23]. Although less common, staphylococcal TSS was also reported in cases of skin and soft tissue infections [24]. Staphylococcal TSS is divided in two categories, menstrual and non-menstrual [25]. Menstrual TSS occurs within two days of a woman’s menstrual period and is usually associated with tampon use. Approximately half of the reported cases are of a non-menstrual nature and are reported in a variety of cases, including surgical wound infections, burns, and cutaneous and subcutaneous lesions. The fatality rate of these infections remains around 5% [26].

Toxic shock presents classically in three phases. The first phase is characterized by fever, myalgia, headache, and chills. Nausea, vomiting, and diarrhea may also be present. The second phase expands to systemic manifestations, such as tachycardia, tachypnea, and high fever. In STSS, pain is present in the affected limb, abdomen or thorax. The third phase is characterized by circulatory shock accompanied by multi-organ failure [27]. Both, STSS and staphylococcal TSS are superantigen-driven diseases. However, STSS is a result of an invasive infection (e.g., NSTI), while staphylococcal TSS is secondary to a localized infection (e.g., infections of postsurgical or postpartum injuries, burns, soft tissue injuries, and focal infections) [28]. Superantigens (SAgs) are recognized as key exotoxins mediating the systemic excessive inflammatory response of the host [29]. To date, 26 staphylococcal and 11 streptococcal SAgs were identified [30,31]. S. aureus SAgs include the toxic shock syndrome toxin 1 (TSST-1), staphylococcal enterotoxins (SEs) A-E and G-I, and SE-like (SEl) SAgs J-Z [31,32]. The SEs are defined by their emetic activity, while SEls lack this activity or have not been tested yet [32]. TSST-1 was among the first SAgs to be associated with staphylococcal TSS [33]. Streptococcal SAgs include streptococcal pyrogenic exotoxins (Spe) A, C, G-M, streptococcal superantigen (SSA), and streptococcal mitogenic exotoxin Z (SmeZ) [30].

For many years, SAgs were known as pyrogenic toxins based on their common pyrogenic activity [34]. Marrack and Kappler suggested the term superantigen, to emphasize the stimulatory capacity of these exotoxins on T cells [35]. SAgs bind without prior cellular processing to α- and/or β-chains of the major histocompatibility complex (MHC) class II molecules on antigen-presenting cells (APCs) and to the variable β-chains on the T-cell receptor (TCR; Figure 2) [36]. In addition, SAgs can also bind a co-stimulatory molecule CD28 and its ligand CD86 (B7-2) [37,38]. Once the fine MHC-peptide specificity of T cells is bypassed, these interactions result in a massive cytokine storm, including tumor necrosis factor (TNF), interferon (IFN)-γ, interleukin (IL)-1, IL-2, IL-6, CXCL8, CCL2, and CCL3 [39].

The majority of the SAg studies are confined to systemic effects and only a limited number of studies investigated SAg-driven events at the deep tissue site [19,40]. A recent study showed that staphylococcal SAgs TSST-1, SEB, and SEC facilitate the attraction of the adaptive immune system to the local environment through their binding to CD40 on human vaginal epithelial cells [41]. The data suggest that, especially in cases of menstrual TSS, SAgs facilitate infections by the disruption of mucosal barriers and subsequently stimulates chemokine production [41]. Thus, the data indicate that SAg-driven activation of T cells may induce the abundance of homing receptors and thereby promote the migration of activated T cells to the skin or mucosal surfaces. Subsequently, these events lead to exacerbated inflammation of the infected tissue.
4. Pore-Forming Toxins

Pore-forming toxins are a class of bacterial virulence factors that disrupt eukaryotic membrane barriers, cause cell lysis, and have immuno-modulatory functions. In this section, we discuss major GAS and staphylococcal pore-forming toxins and their potential implications in NSTIs.

4.1. GAS Pore-Forming Toxins

Nearly all clinical GAS isolates secrete a potent hemolysin streptolysin S (SLS) [42]. SLS is a small (2.7 kDa) peptide with the ability to lyse red blood cells, which is typically visualized as a zone of clearance around GAS colonies on blood agar plates, a process called β-hemolysis [43]. SLS is encoded by the SLS-associated gene (sag) locus consisting of nine genes (sagABCDEFGHI) [44]. The sagA gene encodes the premature form of SLS, while the others are required for post-translational modification, processing, and export of the mature SLS [44,45]. SLS is cytolytic only when associated with the bacterial cell surface or carrier molecules [46] and it targets primarily red blood cells, platelets, subcellular organelles, and leukocytes [47–49]. It has been suggested that SLS accumulates in cell membranes of eukaryotic cells leading to pore formation and irreversible osmotic lysis of the cells [50], but the exact mechanism is not yet fully understood. A recent study by Higashi and colleagues showed that red blood cell hemolysis by GAS is caused by interactions of SLS with the major erythrocyte anion exchange protein band 3 leading to an osmotic change characterized by a rapid influx of Cl⁻ ions [43]. This finding led the authors to hypothesize that SLS might disrupt similar anion channels in other cell types such as leukocytes, keratinocytes, and endothelial cells [43]. In vivo studies have demonstrated that SLS is a crucial virulence factor in GAS NSTIs [45]. SLS-negative mutants were less virulent in a mouse skin infection model as compared to parental wildtype strains [51], suggesting that SLS expression is detrimental for the pathogenesis of destructive infections. SLS facilitates the translocation of GAS across the epithelial barrier through direct cleavage of junctional proteins occludin and E-cadherin (Figure 3) [52]. In addition, direct damage to keratinocytes is guaranteed via the induction of pyroptosis [53]. An out-of-proportion pain is a critical feature of NSTIs at early stage of infection [1]. A recent study has discovered that SLS activates sensory neurons to produce pain [54]. This leads to a
release of neuropeptides that suppress the recruitment of neutrophils to the site of infection and allows the bacteria to spread [54]. Once deeper layers are reached, the direct cytotoxicity of SLS towards a variety of cells residing within the skin tissue and feeding vessels provokes neutrophil influx and further contributes to tissue damage by synergizing with neutrophil-derived factors [35]. Moreover, SLS actively destroys neutrophils which are recruited to the site of infection [56]. This contributes to a negative outcome in patients in two ways; (i) reduced numbers or lack of neutrophils in the infected tissue is an unfavorable prognostic sign in GAS NSTIs [57] and (ii) neutrophil derived effector molecules contribute to hyper-inflammation and tissue damage [19,58,59]. It has also been suggested that SLS impairs the phagocytic clearance of bacteria and further synergizes with other streptococcal virulence factors, such as Streptolysin O (SLO) and the M-protein to augment tissue injury [60]. Failed clearance of the pathogen in deeper tissue layers allows the bacteria to spread and become systemic. In addition, the bacteria can form biofilm, a recently discovered finding in patients suffering from GAS NSTIs [61]. In line with this discovery, Vajjala and colleagues have shown that both streptolysins (SLS and SLO) are required for inducing endoplasmic reticulum stress in the host which, in turn, promotes GAS invasiveness into deeper tissue and biofilm formation [62].

**Figure 3.** Streptolysin S (SLS) mediated tissue pathology. Group A streptococci (GAS) translocate through the epithelium via cleavage of the junction proteins or direct damage. Once deeper layers are reached SLS stimulates neurons to release calcitonin gene-related protein (CGRP), which inhibits the recruitment of neutrophils. In addition, direct damage of neutrophils, monocytes, and macrophages impairs phagocytic clearance of the bacteria and contributes further to tissue damage. Failed clearance of the pathogen results bacterial dissemination and biofilm formation.

SLO is a 57 kDa oxygen-sensitive, cholesterol-dependent cytolysin, which is encoded by the highly conserved slo gene [63]. SLO targets several eukaryotic cells, including macrophages, neutrophils,
epithelial cells, and endothelial cells [63]. It disrupts cytoplasmic membrane integrity through pore formation, thereby inducing cell death through pyroptosis [64], apoptosis [65], and necrosis [66]. Despite its cytolytic activity, SLO is able to suppress crucial neutrophil functions at early stages of infection, including migration, oxidative burst, degranulation, release of other pro-inflammatory mediators, and formation of neutrophil extracellular traps (NET) [67]. In addition, SLO contributes to impaired phagocytic clearance of GAS, thereby enhancing bacterial virulence in murine infection models [68]. Zhu and colleagues have shown that a GAS mutant lacking slo gene was significantly attenuated in a murine soft tissue infection model [69]. SLO expression is regulated by the two-component system CovR/S, which is known to regulate the expression of up to 15% of the GAS genome [70]. Tissue passage of GAS selects for covR/S mutations resulting in an upregulated SLO expression and further systemic dissemination of the bacteria [71,72]. In addition, Sumby and colleagues have shown that a frameshift mutation in the covS gene results in an up-regulation of slo transcripts [73]. Furthermore, strains with a non-functional CovR/S TCS were characterized by a higher secretion of SLO, suggesting that CovR/S acts as a repressor of several virulence relevant genes including slo [73]. In line with this, it was demonstrated that bacterial isolates derived from invasive human infections show higher SLO activity as compared to isolates from non-invasive infections [74]. Also, antimicrobial peptide LL-37, which is highly abundant in necrotic tissue [75], contributes through the CovR/S system to upregulation of SLO expression and promotes resistance of GAS to killing by human epithelial cells, neutrophils, and macrophages [76]. Moreover, LL-37 promotes vesicle formation by GAS, which contain SLO among other virulence factors, further contributing to GAS pathogenesis [77].

4.2. Staphylococcal Pore-Forming Toxins

Alpha toxin (also referred as Hla or Hemolysin-α) is a 33.3 kDa water soluble monomer and is secreted by approximately 95% of S. aureus isolates [78]. NSTI-associated CA-MRSA strains tend to express higher levels of this protein as compared to hospital-acquired (HA) MRSA strains [79,80]. Alpha toxin lyses human platelets, endothelial cells, epithelial cells, keratinocytes and leukocytes in two different ways [81]. First, high amounts of the secreted monomeric components integrate via the binding of phosphatidylcholine or sphingomyelin and cholesterol into the membrane of target cells [82,83]. The resultant heptamer structure subsequently leads to the pore formation and lysis of the cells [84]. Second, at lower concentrations, alpha toxin binds A Disintegrin and Matalloprotease 10 (ADAM10) [85] leading to the induction of catalytic activity of the receptor. ADAM10 is a eukaryotic cell surface protease, which is expressed by keratinocytes, endothelial cells, and platelets [86–89] and whose substrates are members of the notch, ephrin, and cadherin families [87,90–92]. The loss of the adherence junctions of the epithelium, e.g., due to the cleavage of E-cadherin, disrupts the epithelial barrier function [93]. Alpha toxin induced pore formation and the resulting Ca^{2+} influx further enhance ADAM10 activity [92,94].

Unlike α-toxin, leukocidins consist of two components and are hetero-oligomeric. Woodin demonstrated for the first time the bi-component composition through the fractionation of the Panton-Valentine Leukocidin (PVL) [95]. Using ion-exchange chromatography, it was shown that PVL consists of a subunit F and S representing fast and slow fractions, respectively [95]. This study showed that the two subunits must be combined to reach the maximum cytolytic activity [95]. The assembled leukocidins are octamers consisting of four F and four S subunits [96]. Except of LukAB, which either dimerizes after secretion or is released as a dimer [97,98], the S subunits bind to specific host cell receptors and induce conformational changes to allow dimerization with F subunits, followed by oligomerization of the dimers to form a pre-pore [99]. To date, seven bi-component leukocidins, namely PVL, LukAB, LukED, HlgAB, HlgBC, LukMF’, and LukPQ are described in S. aureus (Table 1).
In this review, we limit our discussion to only five, as LukMF’ and LukPQ are circulating in S. aureus stains infecting non-human hosts [100,101]. Leukocidins kill human cells and/or modulate the host cell signaling. At higher concentration, the formation of pores ultimately results in cell death. PVL, HlgAB, HlgCB, and LukAB activate the NOD-, LRR- and pyrin domain containing 3 (NLRP3) inflammasome in monocytes and macrophages [102–105]. Following NLRP3 activation, caspase 1 triggers a pro-inflammatory response and induces pyroptosis [102–106]. At lower toxin concentrations, leukocidins can alter the activation of neutrophils [107,108], trigger the formation of NETs [109], and alter the intracellular signaling in macrophages and neutrophils [102,104,106].

PVL, encoded by the genes lukF-PV and lukS-PV on bacteriophages [110], was predominantly found (77–100%) in CA-MRSA strains [111,112], which were isolated from skin and soft tissue infections [113, 114]. In contrast, less than 3% of colonizing S. aureus strains have the PVL genes [115]. It has been difficult to investigate the role of PVL in human infectious diseases. Due to receptor specificity, murine models have been proven to be unreliable to study PVL functions [116]. In contrast, rabbit models have demonstrated to be a useful tool to study diseases, such as necrotizing pneumonia [116]. However, rabbit studies confined to the role of PVL in skin infections contradict each other. While Lipinska and colleagues showed that PVL contributes to tissue pathology in the early stages of infection [117], others could not detect a role of PVL in NSTIs [118]. In contrast to animal models, using a panel of monoclonal antibodies against transmembrane proteins expressed by human neutrophils and macrophages, Spaan and colleagues showed that the human C5a receptors 1 and 2 (C5aR1 and C5aR2) are able to bind the S subunit of PVL and facilitate pore formation [108]. In addition, genome wide CRISPR-Cas9 screen of U937 cells identified human CD45 as a receptor for the F subunit of PVL [119]. CD45 is expressed on all nucleated hematopoietic cells, including T cells, B cells, and cells of the myeloid lineage [120].

LukAB is a recently discovered leukocidin. Apart from its release into surrounding tissue, it is also present on bacterial surface [98]. The majority of S. aureus strains harbor the genes lukAB [121], but three out of ten strains fail to express and secrete the protein [122]. The role of LukAB in infections remains elusive. Ex vivo studies showed that LukAB kills human neutrophils by direct interaction with the α-subunit of the αM/β2 integrin (CD11b) [123]. In addition, LukAB can synergize with PVL resulting in cytolytic activity towards monocytes, dendritic cells, and neutrophils [98,104].

| Leukocidin | Other Names | Receptors | Human Cell Targets |
|------------|-------------|-----------|-------------------|
| PVL        | PVL-LukSV   | C5aR1     | Neutrophils       |
|            |             | C5aR2     | Monocytes         |
|            |             |           | Macrophages       |
| LukAB      | LukGH       | CD11b     | Neutrophils       |
|            |             |           | Monocytes         |
|            |             |           | Macrophages       |
|            |             |           | Dendritic cells   |
| LukED      |             | CCR5      | T cells           |
|            |             | CXCR1     | Neutrophils       |
|            |             | CXCR2     | Monocytes         |
|            |             | DARC      | Dendritic cells   |
|            |             |           | Erythrocytes      |
| HlgAB      | γ-hemolysin | CXCR1     | Neutrophils       |
| γ-toxin    |             | CXCR2     | Monocytes         |
|            |             | CCR4      | Macrophages       |
|            |             | DARC      | Erythrocytes      |
| HlgCB      | Leukocidin  | C5aR1     | Neutrophils       |
| γ-hemolysin|             | C5aR2     | Monocytes         |
| γ-toxin    |             |           | Macrophages       |
LukED is another recently discovered leukocidin. Epidemiological studies showed that about 99% of CA-MRSA strains contain the *lukED* locus, whereas MSSA strains were less likely to contain the genes (44%–77%) [115]. CCR5 was identified as a first LukED receptor by screening the susceptibility of different human cell types, including T cells, macrophages, and dendritic cells [124]. Further analysis identified chemokine receptors CXCR1 and CXCR2 as LukED receptors on neutrophils and monocytes, which were not expressing CCR5 [125]. Together with HlgAB, LukED belongs to the most potent hemolytic leukocidins against human erythrocytes [126]. Both leukocidins target Duffy antigen receptor for chemokines (DARC) to lyse erythrocytes, which, in turn, contributes to *S. aureus* growth due to iron release [126].

γ-Hemolysins (HlgAB and HlgCB) share the same F subunit HlgB, but differ in their S subunit. Both are encoded within the same locus by three genes *hlgABC* [127]. Up to 99% of *S. aureus* strains associated with human colonization express both hemolysins [128]. HlgAB exhibits cytolytic activity towards human red blood cells and leukocytes [129,130], whereas HlgCB is primarily leukotoxic and exhibits only limited cytolytic activity towards red blood cells [131]. As mentioned above, red blood cell lysis is assured through the HlgAB and DARC interaction [126]. In addition, CXCR1, CXCR2, CXCR4, and CCR2 were identified as HlgAB receptors on human neutrophils and macrophages [131]. In contrast, HlgCB interacts with human neutrophils and monocytes via complement receptors C5aR1 and C5aR2 [131].

Phenol-soluble modulins (PSMs) are another class of staphylococcal pore-forming toxins which were discovered first in *S. epidermidis* in 1999 [132]. Eight years later, PSMs were also identified within *S. aureus* core genome [133]. PSMs are divided in two different subfamilies. PSMα peptides (PSMα1- PSMα4 and δ-hemolysin [Hld]) of short amino acid sequence (20–26) are encoded within the *psmα* operon. PSMβs (PSMβ1 and PSMβ2), which are long peptides (40–44 amino acids) are encoded within the *psmβ* operon [134]. δ-Hemolysin is encoded within the coding sequence of RNAIII [135]. PSM peptides have a strong impact on the capacity of *S. aureus* to cause skin infections [118,133]. Especially, CA-MRSA strains tend to express higher amounts of PSMs as compared to HA-MRSA strains [118,133]. One of the major contributions of PSMs to *S. aureus* pathogenesis is the ability to lyse eukaryotic cells. In contrast to α-toxin and bi-component leukocidins, it is most likely a receptor independent process [136]. PSMα peptides have the strongest ability to lyse erythrocytes and leukocytes, Hld has moderate cytolytic activity, and PSMβ peptides are not cytolytic [137]. Several studies have demonstrated that PSMα peptides facilitate killing of osteoblasts [138] and neutrophils after phagocytosis [139,140]. At sublytic concentrations, PSMα4 initiates pro-inflammatory responses, including chemoattraction and activation of neutrophils leading to a release of CXCL8 [133,136] and heparin-binding protein, which further induces vascular leakage [141]. PSMα1, PSMα3, and Hld can also induce mast cell degranulation [142] and stimulate IL-10 production by human dendritic cells, which in turn suppresses secretion of pro-inflammatory cytokines [143]. Consequently, these dendritic cells favor priming of regulatory T cells with suppressor function, thereby impairing the Th1 response [143]. Recently, it was also shown that PSMα triggers cutaneous inflammation [144]. The release of IL-1α and IL-36α by keratinocytes drives IL-17 production by γδ T cells and type 3 innate lymphoid cells (ILC3) leading to neutrophil recruitment to the site of infection [144].

Although several virulence factors are implicated in contributing towards fulminant NSTIs, each exotoxin might play a certain redundant and/or non-redundant role in eliciting tissue damage and inflammation. During NSTIs, several of these secreted virulence factors might be co-expressed and in turn collectively contribute towards fulminant infections. The cell specificity of these several toxins may play a major role in the coordinated action of the toxin-induced tissue damage. For example, α-toxin and PVL can synergize. Alpha toxin induces the direct cytolytic effect towards epithelial cells which will result in CXCL8 release and subsequent neutrophil chemotaxis. The presence of PVL will activate and lyse recruited neutrophils exacerbating the tissue damage [106,145]. This phenomenon has been mainly shown in experiments using lung epithelial cells. A similar mode of action might also
be relevant in NSTIs. Similarly, both PVL and LukAB can individually cause neutrophil lysis, but their cytotoxic effect is further enhanced when combined together [98,106]. In addition, SAg-translocation (e.g., TSST-1) is augmented by α-toxin and leukocidins which further enhances inflammation of the epithelium and contributes towards epithelial barrier disruption [146,147]. Although this coordinated effect of toxin synergism has not been empirically tested during NSTIs, focusing only on individual toxins can definitely obscure the co-operative actions during infection. Hence, further studies focusing on toxin synergisms during NSTIs need to be conducted. Potentially, these combined effects on specific cell types can amplify tissue pathology to the benefit of the invading bacteria and may define the disease severity and clinical outcome.

5. Proteases and Other Immune-Modulatory Toxins

Proteases are secreted virulence factors which promote establishment of infection through damage of barriers. They inhibit transmigration of immune cells to the site of infection and suppress their function. In this section, we discuss major streptococcal and staphylococcal proteases and their implication in NSTIs.

5.1. Streptococcal Proteases and Other Toxins

Despite its name, streptococcal pyrogenic exotoxin B (SpeB), SpeB is neither pyrogenic nor an exotoxin. SpeB is a cysteine protease and one of the first proteases identified in GAS [148]. The speB gene is highly conserved in all GAS strains [149]. The gene encodes a zymogen of 40 kDa that is autocatalytically cleaved into a mature 28 kDa protein [150]. SpeB cleaves a broad spectrum of streptococcal and human host proteins. On the bacterial site, SpeB is able (i) to remove proteins from the surface, which includes M-protein, fibronectin-binding proteins, and C5a peptidase [151–153] and (ii) to hydrolyze secreted proteins, such as streptokinase, EndoS, SLO, and SAgS [154–157]. On the host site, SpeB cleaves IgG into Fc and Fab fragments and degrades IgA, IgM, IgD, and IgE [158]. The cleavage of IgG results in impaired opsonophagocytosis and increased survival of GAS in human blood [159]. Further, SpeB cleaves components of the complement activation pathway. Kuo and colleagues demonstrated that C3b is cleaved by SpeB leading to impaired phagocytic killing of bacteria by neutrophils [160]. In support of this, Terrao and colleagues detected only degraded C3b fragments in sera of patients diagnosed with STSS [161]. Moreover, SpeB degrades a wide range of chemokines, including, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL16, CCL20, XCL1, and CX3CL1 [162] and cleaves pro-IL-1β into biologically active IL-1β [163]. It was suggested that IL-1β, which activates the NLRP3 inflammasome acts as a sensor of intracellular proteolytic activity of SpeB [164]. Moreover, IL-1β pathway plays a key role in modulating susceptibility of the host to GAS NSTIs [165]. SpeB also interferes with coagulation and anticoagulation pathways by degrading fibrinogen and plasmin, respectively [166,167] and contributes to tissue pathology via the degradation of extracellular matrix proteins and the activation of matrix metalloproteases [168,169].

Although SpeB shows such a broad spectrum of substrates, its role in invasive GAS infections is still controversial. The speB gene can be found in isolates from all types of diseases [170,171]. Some studies show that SpeB is readily detectable in patients’ sera and tissues [61,172]. Others demonstrate that SpeB amounts and activity produced by isolates from non-severe cases are higher as compared to isolates from severe cases [173]. However, low anti-SpeB antibody titers have been associated with severe diseases [174]. This controversy continues also in interpretation of the results generated from mice models. While some authors report that SpeB contributes to disease severity, mortality, bacterial dissemination, and tissue damage [175–178], others show that speB-deficient strains are as virulent as the parental wild type strains [179,180]. Loss of SpeB expression through mutations in coeR/S or ropB is believed to trigger a hyper-virulent phenotype of bacteria [72]. However, human tissues from NSTIs cases are strongly positive for SpeB [61,75]. As recently shown, most likely it is a mixed population of SpeB-positive and SpeB-negative clones contributing to tissue pathology and disease severity [61].
Immunoglobulin degrading enzyme of *S. pyogenes* (IdeS) is a 35 kDa secreted cysteine protease which hydrolyses four subclasses of human IgG [181]. As a consequence, bacterial bound IgGs that are cleaved by IdeS lack IgG-Fc receptor and complement binding/activation capability. Apart from its implications as an important anti-phagocytic virulence factor [182], the role of IdeS in NSTIs is not yet clear.

GAS express two major subtilisin-like serine proteases with immunomodulatory functions, C5a peptidase (ScpA) and SpyCEP. ScpA contains an LPXTG motif which facilitates anchoring of the protein to the bacterial cell wall [183]. Until recently, the human anaphylatoxin C5a was reported as the only substrate for ScpA [184]. The cleavage of C5a results in impaired neutrophil activation and recruitment to the site of infection [185]. Recently, Lyskey and colleagues identified C3 and C3a as novel substrates for ScpA [186]. Cleavage of C3a leads to impaired human neutrophil activation, phagocytosis, and chemotaxis, while cleavage of C3 generated C3a and C3b fragments with impaired functions [186]. SpyCEP is a 180 kDa, surface-exposed, subtilisin-like serine protease that helps GAS to disseminate in soft tissue [187,188]. SpyCEP is highly expressed in vivo [189] and cleaves CXC chemokines, including CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, and CXCL8 [187,188,190], which results in an impaired chemoattraction of eosinophils, neutrophils, and monocytes to the site of infection [189,191]. Moreover, SpyCEP promotes resistance to phagocytic clearance of bacteria by reducing formation of NETs [189]. Recently it was also shown that functional SpyCEP is detrimental for invasion of human epithelial and endothelial cells and for biofilm formation [192].

NAD-glycohydrolase (NADase) is encoded by the *nga* gene and is co-transcribed with the *slo* gene [193]. NADase cleaves NAD in mammalian cells, thereby promoting cytotoxicity through the depletion of energy sources [194]. Several in vivo and in vitro studies have demonstrated synergistic toxicity by SLO and NADase in GAS infections [69,193]. A recent study suggests that binding of NADase to SLO stabilizes both toxins, thereby increasing host cell toxicity [195].

SpyA is a 25 kDa surface exposed C3-like ADP-ribosyltransferase which catalyzes the transfer of an ADP ribose moiety of NAD+ to target proteins [196–198]. It is believed that SpyA modifies actin, vimentin, and tropomyosin to disrupt cytoskeletal structures and promote colonization of the host [196]. In addition, SpyA induces pyroptosis in macrophages, resulting in a release of IL-1β, which in turn enhances bacterial clearance [199].

Streptokinase (Ska) is a plasminogen activator protein which non-enzymatically converts plasminogen to proteolytically active plasmin [200]. To date, Ska has been found in all GAS isolates. The molecule is comprised of three domains (α, β, and γ) and three distinct *ska* alleles, type 1, 2a, and 2b have been described [201]. The majority of GAS strains isolated from skin infections are harboring type 2b *ska* allele [201]. Although Ska activates plasminogen, it is not a protease. GAS cover their surface via different surface anchored or surface associated virulence factors with plasminogen, which, in turn, leads to acquisition of streptokinase [202,203]. The Ska-plasminogen interaction leads to exposure of an active site in the complex, which results in a proteolytical conversion of plasminogen to plasmin [204,205]. Due to host-specificity of Ska, GAS are exclusively human pathogens, no differences in virulence between wildtype and *ska*-deficient GAS mutants are seen in murine infection models [206]. In humanized transgenic mice, expressing human plasminogen, the mortality of mice infected with *ska*-mutant is largely abrogated [207]. In line with this, the SpeB-negative M1T1 GAS variant 5448AP expresses higher levels of Ska as compared to the parental strain 5448 and shows higher surface plasminogen acquisition resulting in hyper-virulence in a subcutaneous infection model of humanized transgenic mice [72].

5.2. *Staphylococcal Proteases and Other Toxins*

Staphylococcal cysteine proteases are papain-like proteases that belong to the C47 family of cysteine peptidases. They can directly or indirectly damage the epithelium as well as connective tissue [208]. Two cysteine proteases, staphopain A (ScpA) and staphopain B (SspB), were identified in *S. aureus*. ScpA is a 20 kDa protein, which auto-activates upon release into environment [209]. Its broad
spectrum of substrates includes collagen, elastin, fibronectin, fibrinogen, and kininogen [210,211]. In addition, ScpA blocks CXCR2 on neutrophils via cleavage of the N-terminal domain, making neutrophils unresponsive to activation by all CXCR2 ligands [212]. Moreover, this cleavage results in impaired neutrophil migration towards CXCR2 chemokines [212]. SspB is a 20 kDa peptidase, which is structurally related to ScpA [209]. SspB cleaves CD11b on monocytes and neutrophils resulting in an atypical cell death [213]. Moreover, SspB blocks phagocytosis of \textit{S. aureus} by neutrophils and monocytes and represses their chemotactic activity by a yet unknown mechanism [214].

The group of staphylococcal serine proteases encloses three major classes: the SspA (or V8 protease), epidermin leader peptide processing serine protease (EpiP), and exfoliative toxins (ETs). V8 protease is secreted as an inactive precursor and requires aureolysin (Aur) for its maturation [209]. The mature V8 protease degrades the Fc region of immunoglobulins leading to impaired interaction of immune effector cells with the antigen [215]. In skin infections, V8 protease disrupts the structure of the \textit{stratum corneum} but does not cause epidermal hyper-proliferation or inflammatory cell infiltration [216]. The role of EpiP in \textit{S. aureus} pathogenesis is not fully understood. EpiP is a subtilisin-like serine protease that cleaves collagen [217]. Mice vaccinated with EpiP were protected from subcutaneous \textit{S. aureus} infection [217]. As mentioned above, its structural homologue in \textit{S. pyogenes}, SpyCEP, inactivates CXCL8 and impairs the recruitment of neutrophils to the site of infection [187,189]. However, whether EpiP has similar pathogenic mechanisms remains to be investigated. The third class of serine proteases are the epidermolytic ETs. Although not involved in severe skin infections, ETs can cause breakage of the upper layers of the skin [218]. Four ETs, namely ETA, ETB, ETC, and ETD are known so far [218]. However, ETA and ETB are implicated in human skin infections [219], while ETC and ETD are more related to non-human hosts. Both, ETA and ETB cleave desmoglein 1, a glycoprotein responsible for cell-cell adhesion of the keratinocytes in \textit{stratum granulosum} without affecting E-cadherin [220]. In addition to serine proteases, \textit{S. aureus} secretes six serine protease-like proteins (SplA-SplF) [222], which show amino acid homology with SspA and ETs [222]. In contrast to other serine proteases, Spls are mainly implicated in allergic airway reactions such as asthma [223].

Aureolysin (Aur) belongs to the family of zinc-dependent metallopeptidases [224]. In vitro, it was shown that Aur cleaves \(\alpha\)-1-protease inhibitor, which is responsible for regulation of neutrophil elastase [225]. In line with this, Burlak and colleagues demonstrated that Aur is expressed within phagocytic vacuoles of human neutrophils [226]. Moreover, Aur can cleave the antimicrobial peptide LL-37 [227] and complement component C3 to C3b [228]. As a result, \textit{S. aureus} is poorly opsonized leading to attenuated phagocytosis and bacterial killing [228].

Staphylokinase (SAK) is a secreted and cell surface associated virulence factor of staphylococci and is structurally unrelated to streptokinase [229]. Especially clinical \textit{S. aureus} isolates of skin and mucosal origin express high levels of SAK [230]. SAK stimulates the production of human antimicrobial peptides (LL-37 and \(\alpha\)-defensins), binds, and inactivates their bactericidal properties [231,232]. However, the main SAK activity affects its ability to convert plasminogen to an active proteolytic enzyme plasmin [230]. First, \textit{S. aureus} binds plasminogen via surface expressed proteins (e.g., FnBPA and FnBPB) and second, SAK activates plasminogen to plasmin, thereby creating a bacteria-bound serine protease activity [233]. These events enable the bacteria to degrade immunoglobulin G (IgG) and C3b, thereby contributing to immune evasion [234].

5.3. Two Component Systems and Exotoxin Regulation

During bacterial infections the regulation of exotoxins is mediated by a complex network which incorporates environmental signals towards coordinated responses against host microenvironment. Two component systems (TCS) are one such mechanism adopted by bacteria. An external signal activates the membrane bound histidine kinase. This induces auto-phosphorylation and downstream activation of a response regulator by its phosphorylation. The binding of the regulator to specific DNA sequence results in its gene expression. Most important and well-studied TCS are AgrAC and SaeRS in
S. aureus and CovR/S in GAS strains. Both are known to regulate the virulence factors that mitigate the host responses during fulminant NSTIs [235,236].

Differential gene regulation of exotoxins by TCS determines the specificity of toxin gene expression at the site of infection. S. aureus exotoxins are upregulated in a growth density dependent manner during NSTIs [237,238]. Furthermore, leukocidins are found to be upregulated during NSTIs [239]. These virulence factors are mainly regulated by the intracellular effector responses belonging to agr quorum-sensing system including the transcriptional regulators AgrA and RNA III [240–242]. The agr system governs the expression of secreted virulence factors and exotoxins which enhance acute infection and bacterial dissemination [240–242]. Virulence factor production is mainly regulated through two pathways: (i) RNAIII-dependent synthesis of exotoxins and inhibition of cell surface factors and (ii) an RNAIII-independent, AgrA-mediated production of PSMs and metabolic genes. However, mutations in the agr-operon rendering dysfunctional Agr system are associated with adaptation of the bacteria to host environment and inducing a more persistent phenotype [243]. Such Agr-defective systems are usually detected in colonizing strains and in strains isolated from patients diagnosed with endocarditis or bacteremia [244]. Similarly, a point mutation in the agrC region was associated with cytotoxic versus colonizing properties of S. aureus phenotypic variants causing skin and soft tissue infections [245]. These data further support the importance of virulence regulation and its impact on clinical presentation. Enhanced virulence expression mediated by active Agr system is usually detected during severe invasive and acute infections such as NSTIs [246], whereas agr-mutants are usually implicated in causing dormant state and chronic infections, such as endocarditis and osteomyelitis [240]. In addition to the Agr system, the S. aureus exoprotein expression system (SaeRS) plays also an important role in regulating virulence factor production at the tissue site. SaeRS consists of the histidine kinase SaeS and the response regulator SaeR. SaeR activates transcription of the downstream target genes [247]. The activated SaeRS TCS induces the expression of several virulence factors, including α-toxin, β- and γ-hemolysins, PVL, TSST-1, and exfoliative toxins [248,249]. Human neutrophil peptides 1, 2, and 3 (HNP1-3), which are located in azurophilic granules of neutrophils and calprotectin, a cytoplasmic neutrophil peptide, activate the SaeRS system [250]. Therefore, neutrophil-mediated activation may play a pivotal role in exotoxin regulation and toxin production at the tissue site. It was proposed that SaeRS TCS acts downstream of Agr in virulence regulation and toxin production pathways [251]. However, the exact mechanism of a relationship between Agr and SaeRS systems is still not fully understood. Moreover, recent studies have implicated that the Agr and SaeRS are independent systems of toxin regulation [247,252,253].

Recent evidence indicates that GAS invasiveness is instigated by spontaneous mutations of the CovR/S TCS [61,72,73,254]. CovR/S is a negative transcriptional regulator of around 15% of the GAS genome. It was shown that mice tissue passage of GAS selects for a 7-bp frame-shift mutation in the covS gene encoding the sensor kinase component and this in turn promotes GAS invasiveness [61,72,73]. Since that discovery several investigators reported the role of CovR/S system in severe invasive infections. Mutations in that particular region result in the upregulation of several secreted virulence factors, including a bacteriophage-encoded DNase [73], SLO [71], and SpyCEP [73]. In addition, dysfunctionality of CovR/S results in loss of SpeB expression. Whether the loss of SpeB and/or enhanced expression of other secreted virulence factors are beneficial for the bacteria or not, was discussed earlier in this article. However, the role of the host tissue micro-environment and the availability of nutrients, which influence the expression of response regulators during NSTIs is still not fully understood. Future studies focused towards understanding the interplay between the signaling pathways will be essential to better understand the physiological significance of toxin expression in the context of host tissue micro-environment during NSTIs.

6. Treatment

The management of NSTI patients includes fluid resuscitation, support of failing organs, rapid surgical debridement of infected tissue, broad spectrum antibiotics, and adjuvant intravenous
polyspecific immunoglobulin G (IVIG) and/or hyperbaric oxygen (HBO) therapy [1,2,255–257]. Aggressive tissue debridement guarantees elimination of the necrotic tissue and the source of infection and exotoxins. Recent studies suggest that early surgical intervention within 24 h post admission significantly improves the survival of patients [258,259]. Survival further increases if debridement is performed even earlier [260,261].

Nearly all GAS are susceptible to penicillin. However, the high bacterial load in the tissue results in most GAS being in the stationary or in biofilm stage, making cell-wall active antimicrobials not always effective [61,262]. Therefore, treatment with clindamycin, a protein synthesis inhibitor, in combination with penicillin is strongly recommended [257]. However, clinical data based on randomized trials are lacking. Clindamycin inhibits production of SAgs [263] and a recent observational study showed that clindamycin improves survival of patients with STSS [264]. Nonetheless, experimental data suggest that sub-inhibitory concentrations of clindamycin enhance expression and activity of SLO in vitro, but suppress the expression of SpeB [265,266]. In addition, the rise of clindamycin resistant GAS strains [267] raises concerns about the benefits of clindamycin treatment.

When MRSA is suspected, i.v. linezolid or daptomycin may be added in preference of vancomycin, as the latter has no effect on exotoxin production [1,257]. In addition, poor tissue penetration of vancomycin lowers its efficacy in severe NSTIs [268]. Linezolid, an oxazolidinone, inhibits bacterial exotoxin production [269] and several studies concluded that linezolid is an effective alternative to vancomycin for treatment of skin infections caused by MRSA [270–272]. In contrast, daptomycin is a cyclic lipopeptide with a distinct mechanism of action. It inserts into the cell membrane of bacteria via phosphatidylglycerol and disrupts membrane integrity by extracting lipids resulting in ion leakage [273]. Overall, inhibitors of toxin production, such as clindamycin, linezolid or rifampicin are commonly recommended for inclusion in antimicrobial treatment of necrotizing infections.

The use of IVIG and HBO as adjunctive therapies is still under debate. Experimental data showed that IVIG neutralizes bacterial exotoxins, including streptococcal and staphylococcal SAgs [274–277], α-toxin [145], bi-component leukocidins [145], and SLO [278] among others. However, clinical studies contradict each other. One of the first studies in seven patients with severe NSTI caused by GAS suggested a beneficial role of IVIG [279]. A prospective observational study conducted in STSS patients showed reduced mortality in patients receiving IVIG, while a sub-analysis of the NSTI patients did not confirm this observation [264]. In line with this, a recent placebo-controlled clinical trial called INSTINCT, showed no benefit of the IVIG use in NSTIs [255]. The latest systematic review and meta-analysis of the previous single randomized and four nonrandomized studies revealed that administration of IVIG to clindamycin treated patients is associated with a significant reduction in mortality [280]. Same contradiction applies to adjunctive HBO treatment. Two recent studies concluded that HBO treatment is associated with significant reduction in mortality in NSTIs [7,281]. Nevertheless, a systematic literature review of 57 studies revealed that HBO is not useful for the treatment of NSTIs [282]. Currently, a study delineating the effects of HBO on biomarkers in NSTIs is being performed in Denmark [283].

7. Conclusions

NSTIs are rapidly progressing, life-threatening necrotic infections of any layer of the soft tissue compartment. The underlying mechanisms of these infections are poorly understood. GAS and S. aureus are equipped with an arsenal of virulence factors that contribute to disease pathogenesis. In NSTIs, there is a clear correlation between exotoxin production at the site of infection and tissue pathology and systemic toxicity. Therefore, secreted virulence factors, including SAgs, pore-forming toxins, and immunomodulatory proteases, are attractive targets for therapeutic approaches. However, further understanding of mechanistic actions of the exotoxins in vivo and in vitro is needed.

**Author Contributions:** PS. and N.S. conceived the concept of this review article. P.S., S.M.S., and N.S. curated the data, wrote, and edited the manuscript.
**Funding:** Research on bacterial pathogenesis in authors’ laboratories is supported by Deutsche Forschungsgemeinschaft (DFG, German Research Foundation), grant number 407176682 and by Federal Excellence Initiative of Mecklenburg Western Pomerania and European Social Fund (ESF) Grant KoInfekt (ESF_14-BM-A55-0001_16). S.M.S is supported by a grant from the Swedish Society for Medical Research (SSMF) Post-Doctoral research fellowship.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. Morgan, M.S. Diagnosis and management of necrotising fasciitis: A multiparametric approach. *J. Hosp. Infect.* 2010, 75, 249–257. [CrossRef] [PubMed]

2. Anaya, D.A.; McMahon, K.; Nathens, A.B.; Sullivan, S.R.; Foy, H.; Bulger, E. Predictors of mortality and limb loss in necrotizing soft tissue infections. *Arch. Surg.* 2005, 140, 151–157. [CrossRef] [PubMed]

3. Harbrecht, B.G.; Nash, N.A. Necrotizing soft tissue infections: A review. *Surg. Infect. Larchmt.* 2016, 17, 503–509. [CrossRef] [PubMed]

4. Stevens, D.L.; Bryant, A.E. Necrotizing soft tissue infections. *N. Engl. J. Med.* 2017, 377, 2253–2265. [CrossRef] [PubMed]

5. Giuliano, A.; Lewis, F.; Hadley, K.; Blaisdell, F.W. Bacteriology of necrotising fasciitis. *Am. J. Surg.* 1977, 134, 52–57. [CrossRef] [PubMed]

6. Ustin, J.S.; Malangoni, M.A. Necrotizing soft-tissue infections. *Crit. Care Med.* 2011, 39, 2156–2162. [CrossRef] [PubMed]

7. Devaney, B.; Frawley, G.; Frawley, L.; Pilcher, D.V. Necrotising soft tissue infections: The effect of hyperbaric oxygen on mortality. *Anaesth Intensive Care* 2015, 43, 685–692. [CrossRef] [PubMed]

8. Glass, G.E.; Sheil, F.; Ruston, J.C.; Butler, P.E. Necrotising soft tissue infection in a uk metropolitan population. *Ann. R. Coll. Surg. Engl.* 2015, 97, 46–51. [CrossRef] [PubMed]

9. Khamnuan, P.; Chongruksut, W.; Jearwattanakanok, K.; Patumanond, J.; Tantraworasin, A. Necrotizing fasciitis: Epidemiology and clinical predictors for amputation. *Int. J. Gen. Med.* 2015, 8, 195–202.

10. Nordqvist, G.; Wallden, A.; Brorson, H.; Tham, J. Ten years of treating necrotizing fasciitis. * Infect. Dis.* 2015, 47, 319–325. [CrossRef]

11. Miller, L.G.; Perdreau-Remington, F.; Rieg, G.; Mehdi, S.; Perlroth, J.; Bayer, A.S.; Tang, A.W.; Phung, T.O.; Spellberg, B. Necrotizing fasciitis caused by community-associated meticillin-Resistant staphylococcus aureus in los angeles. *N. Engl. J. Med.* 2005, 352, 1445–1453. [CrossRef] [PubMed]

12. Goh, T.; Goh, L.G.; Ang, C.H.; Wong, C.H. Early diagnosis of necrotizing fasciitis. *Br. J. Surg.* 2014, 101, e119–e125. [CrossRef] [PubMed]

13. Chambers, H.F.; Deleo, F.R. Waves of resistance: Staphylococcus aureus in the antibiotic era. *Nat. Rev. Microbiol.* 2009, 7, 629–641. [CrossRef] [PubMed]

14. DeLeo, F.R.; Otto, M.; Kreiswirth, B.N.; Chambers, H.F. Community-associated meticillin-Resistant staphylococcus aureus. *Lancet* 2010, 375, 1557–1568. [CrossRef]

15. Kleven, R.M.; Morrison, M.A.; Nadle, J.; Petit, S.; Gershman, K.; Ray, S.; Harrison, L.H.; Lynfield, R.; Dumyati, G.; Townes, J.M.; et al. Invasive meticillin-Resistant staphylococcus aureus infections in the united states. *JAMA* 2007, 298, 1763–1771. [CrossRef]

16. Carapetis, J.R.; Steer, A.C.; Mulholland, E.K.; Weber, M. The global burden of group a streptococcal diseases. *Lancet Infect. Dis.* 2005, 5, 685–694. [CrossRef]

17. Stevens, D.L.; Tanner, M.H.; Winship, J.; Swarts, R.; Ries, K.M.; Schlievert, P.M.; Kaplan, E. Severe group a streptococcal infections associated with a toxic shock-like syndrome and scarlet fever toxin A. *N. Engl. J. Med.* 1989, 321, 1–7. [CrossRef]

18. Nuwayhid, Z.B.; Aronoff, D.M.; Mulla, Z.D. Blunt trauma as a risk factor for group a streptococcal necrotizing fasciitis. *Ann. Epidemiol.* 2007, 17, 878–881. [CrossRef]

19. Johansson, L.; Thuillin, P.; Low, D.E.; Norrby-Teglund, A. Getting under the skin: The immunopathogenesis of streptococcus pyogenes deep tissue infections. *Clin. Infect. Dis.* 2010, 51, 58–65. [CrossRef]

20. Singer, M.; Deutschman, C.S.; Seymour, C.W.; Shankar-Hari, M.; Annane, D.; Bauer, M.; Bellomo, R.; Bernard, G.R.; Chiche, J.D.; Coopersmith, C.M.; et al. The third international consensus definitions for sepsis and septic shock (sepsis-3). *JAMA* 2016, 315, 801–810. [CrossRef]
21. Kaul, R.; McGeer, A.; Low, D.E.; Green, K.; Schwartz, B. Population-based surveillance for group a streptococcal necrotizing fasciitis: Clinical features, prognostic indicators, and microbiologic analysis of seventy-seven cases. Ontario group a streptococcal study. *Am. J. Med.* 1997, 103, 18–24. [CrossRef]

22. Stevens, D.L.; Bisno, A.L.; Chambers, H.F.; Everett, E.D.; Dellinger, P.; Goldstein, E.J.; Gorbach, S.L.; Hirschmann, J.V.; Kaplan, E.L.; Montoya, J.G.; et al. Practice guidelines for the diagnosis and management of skin and soft-tissue infections. *Clin. Infect. Dis.* 2005, 41, 1373–1406. [CrossRef] [PubMed]

23. Simonart, T. Group a beta-Haemolytic streptococcal necrotising fasciitis: Early diagnosis and clinical features. *Dermatol. Surg.* 2004, 30, 5–9. [CrossRef] [PubMed]

24. DeVries, A.S.; Lesher, L.; Schlievert, P.M.; Rogers, T.; Villaume, L.G.; Danila, R.; Lynfield, R. Staphylococcal toxic shock syndrome 2000–2006: Epidemiologic, clinical features, and molecular characteristics. *PLoS ONE* 2011, 6, e22997. [CrossRef] [PubMed]

25. Wharton, M.; Chorba, T.L.; Vogt, R.L.; Morse, D.L.; Buehler, J.W. Case definitions for public health surveillance. *MMWR Recomm. Rep.* 1990, 39, 273–279.

26. Hajjeh, R.A.; Reingold, A.; Weil, A.; Shutt, K.; Schuchat, A.; Perkins, B.A. Toxic shock syndrome in the united states: Surveillance update, 1979–1996. *Emerg. Infect. Dis.* 1999, 5, 807–810. [CrossRef] [PubMed]

27. Schmitz, M.; Roux, X.; Huttner, B.; Pugin, J. Streptococcal toxic shock syndrome in the intensive care unit. *Ann. Intensive Care* 2018, 8, 88. [CrossRef] [PubMed]

28. Low, D.E. Toxic shock syndrome: Major advances in pathogenesis, but not treatment. *Crit. Care Clin.* 2013, 29, 651–675. [CrossRef] [PubMed]

29. Llewelyn, M.; Cohen, J. Superantigens: Microbial agents that corrupt immunity. *Lancet Infect. Dis.* 2002, 2, 156–162. [CrossRef]

30. Commons, R.J.; Smeesters, P.R.; Proft, T.; Fraser, J.D.; Robins-Browne, R.; Curtis, N. Streptococcal superantigens: Categorization and clinical associations. *Trends Mol. Med.* 2014, 20, 48–62. [CrossRef] [PubMed]

31. Tu, ff

32. Marrack, P.; Kappler, J. The staphylococcal enterotoxins and their relatives. *Pathogens* 2018, 7, 53. [CrossRef] [PubMed]

33. Schlievert, P.M.; Schoettle, D.J.; Watson, D.W. Purification and physicochemical and biological characterization of a staphylococcal pyrogenic exotoxin. *Infect. Immun.* 1979, 23, 609–617. [PubMed]

34. Bohach, G.A.; Fast, D.J.; Nelson, R.D.; Schlievert, P.M. Staphylococcal and streptococcal pyrogenic toxins involved in toxic shock syndrome and related illnesses. *Crit. Rev. Microbiol.* 1990, 17, 251–272. [CrossRef] [PubMed]

35. Marrack, P.; Kappler, J. The staphylococcal enterotoxins and their relatives. *Science* 1990, 248, 705–711. [CrossRef] [PubMed]

36. Siemens, N.; Norrby-Teglund, A. Shocking superantigens promote establishment of bacterial infection. *Proc. Natl. Acad. Sci. USA* 2017, 114, 10000–10002. [CrossRef] [PubMed]

37. Arad, G.; Levy, R.; Nasie, I.; Hillman, D.; Rotfogel, Z.; Barash, U.; Supper, E.; Shpilka, T.; Minis, A.; Kaempfer, R. Binding of superantigen toxins into the cd28 homodimer interface is essential for induction of cytokine genes that mediate lethal shock. *PLoS Biol.* 2011, 9, e1001149. [CrossRef]

38. Levy, R.; Rotfogel, Z.; Hillman, D.; Popugailo, A.; Arad, G.; Supper, E.; Osman, F.; Kaempfer, R. Superantigens hyperinduce inflammatory cytokines by enhancing the b7-2/cd28 costimulatory receptor interaction. *Proc. Natl. Acad. Sci. USA* 2016, 113, E6437–E6446. [CrossRef]

39. Chahita, T.; Geha, R.S. Signal transduction by microbial superantigens via mhc class ii molecules. *Immunol. Rev.* 1999, 131, 43–59. [CrossRef]

40. Norrby-Teglund, A.; Thulin, P.; Gan, B.S.; Kotb, M.; McGeer, A.; Andersson, J.; Low, D.E. Evidence for superantigen involvement in severe group a streptococcal tissue infections. *J. Infect. Dis.* 2001, 184, 853–860. [CrossRef]

41. Schlievert, P.M.; Cahill, M.P.; Hostager, B.S.; Brosnahan, A.J.; Klingelhoht, A.J.; Gourronc, F.A.; Bishop, G.A.; Leung, D.Y.M. Staphylococcal superantigens stimulate epithelial cells through CD40 to produce chemokines. *mBio* 2019, 10, e00214-19. [CrossRef] [PubMed]
42. Yoshino, M.; Murayama, S.Y.; Sunaoshi, K.; Wajima, T.; Takahashi, M.; Masaki, J.; Kurokawa, I.; Ubukata, K. Non-hemolytic streptococcus pyogenes isolates that lack large regions of the sag operon mediating streptolysin s production. J. Clin. Microbiol. 2010, 48, 635–638. [CrossRef] [PubMed]
43. Higashi, D.L.; Biais, N.; Donahue, D.L.; Mayfield, J.A.; Tessier, C.R.; Rodriguez, K.; Ashfeld, B.L.; Luchetti, J.; Ploplis, V.A.; Castellino, F.J.; et al. Activation of band 3 mediates group a streptococcus streptolysin s-based beta-haemolysis. Nat. Microbiol. 2016, 1, 15004. [CrossRef] [PubMed]
44. Nizet, V.; Beall, B.; Bast, D.J.; Datta, V.; Kilburn, L.; Low, D.E.; De Azavedo, J.C. Genetic locus for streptolysin s production by group a streptococcus. Infect. Immun. 2000, 68, 4245–4254. [CrossRef] [PubMed]
45. Molloy, E.M.; Cotter, P.D.; Hill, C.; Mitchell, D.A.; Ross, R.P. Streptolysin S-like virulence factors: The continuing saga. Nat. Rev. Microbiol. 2011, 9, 670–681. [CrossRef] [PubMed]
46. Ginsburg, I. Is streptolysin s of group a streptococci a virulence factor? APMIS 1999, 107, 1051–1059. [CrossRef] [PubMed]
47. Bernheimer, A.W.; Schwartz, L.L. Lysosomal disruption by bacterial toxins. J. Bacteriol. 1964, 87, 1100–1104. [PubMed]
48. Hryniewicz, W.; Pryjma, J. Effect of streptolysin s on human and mouse t and b lymphocytes. Infect. Immun. 1977, 16, 730–733.
49. Keiser, H.; Weissmann, G.; Bernheimer, A.W. Studies on lysosomes. IV. Solubilization of enzymes during mitochondrial swelling and disruption of lysosomes by streptolysin s and other hemolytic agents. J. Cell Biol. 1964, 22, 101–113. [CrossRef]
50. Carr, A.; Sledjeski, D.D.; Podbielski, A.; Boyle, M.D.; Kreikemeyer, B. Similarities between complement-mediated and streptolysin S-Mediated hemolysis. J. Biol. Chem. 2001, 276, 41790–41796. [CrossRef] [PubMed]
51. Betschel, S.D.; Borgia, S.M.; Barg, N.L.; Low, D.E.; De Azavedo, J.C. Reduced virulence of group a streptococcal TN916 mutants that do not produce streptolysin s. Infect. Immun. 1998, 66, 1671–1679. [PubMed]
52. Sumitomo, T.; Nakata, M.; Higashino, M.; Jin, Y.; Terao, Y.; Fujinaga, Y.; Kawabata, S. Streptolysin s contributes to group a streptococcal translocation across an epithelial barrier. J. Biol. Chem. 2011, 286, 2750–2761. [CrossRef] [PubMed]
53. Flaherty, R.A.; Donahue, D.L.; Carothers, K.E.; Ross, J.N.; Ploplis, V.A.; Castellino, F.J.; Lee, S.W. Neutralization of streptolysin S-Dependent and independent inflammatory cytokine IL-1beta activity reduces pathology during early group a streptococcal skin infection. Front. Cell Infect. Microbiol. 2018, 8, 211. [CrossRef] [PubMed]
54. Pinho-Ribeiro, F.A.; Baddal, B.; Haarasma, R.; O’Seaghdha, M.; Yang, N.J.; Blake, K.J.; Portley, M.; Verri, W.A.; Dale, J.B.; Wessels, M.R.; et al. Blocking neuronal signaling to immune cells treats streptococcal invasive infection. Cell 2018, 173, 1083–1097. [CrossRef] [PubMed]
55. Humar, D.; Datta, V.; Bast, D.J.; Beall, B.; De Azavedo, J.C.; Nizet, V. Streptolysin s and necrotizing infections produced by group g streptococcus. Lancet 2002, 359, 124–129. [CrossRef]
56. Miyoshi-Akiyama, T.; Takamatsu, D.; Koyanagi, M.; Zhao, J.; Imanishi, K.; Uchiyama, T. Cytocidal effect of streptococcus pyogenes on mouse neutrophils in vivo and the critical role of streptolysin s. J. Infect. Dis. 2005, 192, 107–116. [CrossRef] [PubMed]
57. Bakleh, M.; Wold, L.E.; Mandrekar, J.N.; Harmsen, W.S.; Dimashkieh, H.H.; Baddour, L.M. Correlation of histopathologic findings with clinical outcome in necrotizing fasciitis. Clin. Infect. Dis. 2005, 40, 410–414. [CrossRef] [PubMed]
58. Johansson, L.; Linner, A.; Sunden-Cullberg, J.; Haggar, A.; Herwald, H.; Lore, K.; Treutiger, C.J.; Norrby-Teglund, A. Neutrophil-derived hyperresistinemia in severe acute streptococcal infections. J. Immunol. 2009, 183, 4047–4054. [CrossRef] [PubMed]
59. Johansson, L.; Norrby-Teglund, A. Immunopathogenesis of streptococcal deep tissue infections. Curr. Top. Microbiol. Immunol. 2013, 368, 173–188. [PubMed]
60. Datta, V.; Myskowski, S.M.; Kwinn, L.A.; Chiem, D.N.; Varki, N.; Kansal, R.G.; Koth, M.; Nizet, V. Mutational analysis of the group a streptococcal operon encoding streptolysin s and its virulence role in invasive infection. Mol. Microbiol. 2005, 56, 681–695. [CrossRef] [PubMed]
61. Siemens, N.; Chakrakodi, B.; Shambat, S.M.; Morgan, M.; Bergsten, H.; Hyldegaard, O.; Skrede, S.; Arnell, P.; Madsen, M.B.; Johansson, L.; et al. Biofilm in group a streptococcal necrotizing soft tissue infections. JCI Insight 2016, 1, e87882. [CrossRef] [PubMed]
62. Vaijala, A.; Biswas, D.; Tay, W.H.; Hanski, E.; Kline, K.A. Streptolysin-induced endoplasmic reticulum stress promotes group a streptococcal host-associated biofilm formation and necrotising fasciitis. Cell Microbiol. 2019, 21, e12956. [CrossRef] [PubMed]

63. Barnett, T.C.; Cole, J.N.; Rivera-Hernandez, T.; Henningham, A.; Paton, J.C.; Nizet, V.; Walker, M.J. Streptococcal toxins: Role in pathogenesis and disease. Cell Microbiol. 2015, 17, 1721–1741. [CrossRef] [PubMed]

64. Keyel, P.A.; Roth, R.; Yokoyama, W.M.; Heuser, J.E.; Salter, R.D. Reduction of streptolysin o (slo) pore-forming activity enhances inflammasome activation. Toxins 2013, 5, 1105–1118. [CrossRef] [PubMed]

65. Timmer, A.M.; Timmer, J.C.; Pence, M.A.; Hsu, L.C.; Ghochani, M.; Frey, T.G.; Karin, M.; Salvesen, G.S.; Nizet, V. Streptolysin o promotes group a streptococcus immune evasion by accelerated macrophage apoptosis. J. Biol. Chem. 2009, 284, 862–871. [CrossRef] [PubMed]

66. Chandrasekaran, S.; Caparon, M.G. The nadase-negative variant of the streptococcus pyogenes toxin nad(+)

67. Uchiyama, S.; Dohrmann, S.; Timmer, A.M.; Dixit, N.; Ghochani, M.; Bhandari, T.; Timmer, J.C.; Sprague, K.; Bubeck-Wardenburg, J.; Simon, S.I.; et al. Streptolysin o rapidly impairs neutrophil oxidative burst and antibacterial responses to group a streptococcus. Front. Immunol. 2015, 6, 581. [CrossRef]

68. Sierig, G.; Cywes, C.; Wessels, M.R.; Ashbaugh, C.D. Cytotoxic effects of streptolysin O and streptolysin s enhance the virulence of poorly encapsulated group a streptococci. Infect. Immun. 2003, 71, 446–455. [CrossRef]

69. Zhu, L.; Olsen, R.J.; Lee, J.D.; Porter, A.R.; DeLeo, F.R.; Musser, J.M. Contribution of secreted nadase and streptolysin o to the pathogenesis of epidemic serotype m1 streptococcus pyogenes infections. Am. J. Pathol. 2017, 187, 605–613. [CrossRef]

70. Graham, M.R.; Smoot, L.M.; Migliaccio, C.A.; Virtaneva, K.; Sturdevant, D.E.; Porcella, S.F.; Federle, M.J.; Adams, G.J.; Scott, J.R.; Musser, J.M. Virulence control in group a streptococcus by a two-component gene regulatory system: Global expression profiling and in vivo infection modeling. Proc. Natl. Acad. Sci. USA 2002, 99, 13855–13860. [CrossRef]

71. Cole, J.N.; Barnett, T.C.; Nizet, V.; Walker, M.J. Molecular insight into invasive group a streptococcal disease. Nat. Rev. Microbiol. 2011, 9, 724–736. [CrossRef] [PubMed]

72. Walker, M.J.; Hollands, A.; Sanderson-Smith, M.L.; Cole, J.N.; Kirk, J.K.; Henningham, A.; McArthur, J.D.; Dinkla, K.; Aziz, R.K.; Kansal, R.G.; et al. Dnase sda1 provides selection pressure for a switch to invasive group a streptococcal infection. Nat. Med. 2007, 13, 981–985. [CrossRef] [PubMed]

73. Sumby, P.; Whitney, A.R.; Graviss, E.A.; DeLeo, F.R.; Musser, J.M. Genome-Wide analysis of group a streptococci reveals a mutation that modulates global phenotype and disease specificity. PLoS Pathog. 2006, 2, e5. [CrossRef] [PubMed]

74. Siemens, N.; Kittang, B.R.; Chakrakodi, B.; Oppegaard, O.; Johansson, L.; Bruun, T.; Mylvaganam, H.; Group, I.S.; Svensson, M.; Skrede, S.; et al. Increased cytotoxicity and streptolysin o activity in group g streptococcal strains causing invasive tissue infections. Sci. Rep. 2015, 5, 16945. [CrossRef]

75. Johansson, L.; Thulin, P.; Sendi, P.; Hertzgen, E.; Linder, A.; Akesson, S.; Low, D.E.; Agerberth, B.; Norrby-Teglund, A. Cathelicidin LL-37 in severe streptococcus pyogenes soft tissue infections in humans. Infect. Immun. 2008, 76, 3399–3404. [CrossRef] [PubMed]

76. Love, J.F.; Tran-Winkler, H.J.; Wessels, M.R. Vitamin d and the human antimicrobial peptide ll-37 enhance group a streptococcus resistance to killing by human cells. mBio 2012, 3, e00394-12. [CrossRef] [PubMed]

77. Uhlmann, J.; Rohde, M.; Siemens, N.; Kreikemeyer, B.; Bergman, P.; Johansson, L.; Norrby-Teglund, A. Ll-37 triggers formation of streptococcus pyogenes extracellular vesicle-like structures with immune stimulatory properties. J. Innate Immun. 2016, 8, 243–257. [CrossRef] [PubMed]

78. Grumann, D.; Nubel, U.; Broker, B.M. Staphylococcus aureus toxins-Their functions and genetics. Infect. Genet. Evol. 2014, 21, 583–592. [CrossRef]

79. DeLeo, F.R.; Kennedy, A.D.; Chen, L.; Bubeck Wardenburg, J.; Kobayashi, S.D.; Mathema, B.; Braughton, K.R.; Whitney, A.R.; Villaruz, A.E.; Martens, C.A.; et al. Molecular differentiation of historic phase-type 80/81 and contemporary epidemic staphylococcus aureus. Proc. Natl. Acad. Sci. USA 2011, 108, 18091–18096. [CrossRef]
80. Tavares, A.; Nielsen, J.B.; Boye, K.; Rohde, S.; Paulo, A.C.; Westh, H.; Schonning, P.; de Lencastre, H.; Miragaia, M. Insights into alpha-Hemolyzin (hla) evolution and expression among staphylococcus aureus clones with hospital and community origin. *PloS ONE* **2014**, *9*, e98634. [CrossRef] [PubMed]
81. Berube, B.J.; Bubeck Wardenburg, J. Staphylococcus aureus alpha-toxin: Nearly a century of intrigue. *Toxins* **2013**, *5*, 1140–1166. [CrossRef] [PubMed]
82. Valeva, A.; Hellmann, N.; Walev, I.; Strand, D.; Plate, M.; Boukhallouk, F.; Brack, A.; Hanada, K.; Decker, H.; Bkhadi, S. Evidence that clustered phosphocholine head groups serve as sites for binding and assembly of an oligomeric protein pore. *J. Biol. Chem.* **2006**, *281*, 26014–26021. [CrossRef] [PubMed]
83. Watanabe, M.; Tomita, T.; Yasuda, T. Membrane-damaging action of staphylococcal alpha-toxin on phospholipid-Cholesterol liposomes. *Biochim. Biophys. Acta* **1987**, *898*, 257–265. [CrossRef]
84. Hildebrand, A.; Pohl, M.; Bkhadi, S. Staphylococcus-aureus alpha-Toxin-Dual mechanism of binding to target-Cells. *J. Biol. Chem.* **1991**, *266*, 17195–17200. [PubMed]
85. Wilke, G.A.; Bubeck Wardenburg, J. Role of a disintegrin and metalloprotease 10 in staphylococcus aureus alpha-hemolysin-Mediated cellular injury. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 13473–13478. [CrossRef] [PubMed]
86. Colciaghi, F.; Borroni, B.; Pastorino, L.; Marcello, E.; Zimmermann, M.; Cattabeni, F.; Padovani, A.; Di Luca, M. [alpha]-secretase adam10 as well as [alpha]apps is reduced in platelets and csf of alzheimer disease patients. *Mol. Med.* **2002**, *8*, 67–74. [CrossRef] [PubMed]
87. Maretzky, T.; Reiss, K.; Ludwig, A.; Buchholz, J.; Scholz, F.; Proksch, E.; de Strooper, B.; Hartmann, D.; Saftig, P. Adam10 mediates e-cadherin shedding and regulates epithelial cell-Cell adhesion, migration, and beta-Catenin translocation. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 9182–9187. [CrossRef]
88. Maretzky, T.; Scholz, F.; Koten, B.; Proksch, E.; Saftig, P.; Reiss, K. Adam10-Mediated e-Cadherin release is regulated by proinflammatory cytokines and modulates keratinocyte cohesion in eczematous dermatitis. *J. Investig. Dermatol.* **2008**, *128*, 1737–1746. [CrossRef]
89. Schulz, B.; Pruessmeyer, J.; Maretzky, T.; Ludwig, A.; Blobel, C.P.; Saftig, P.; Reiss, K. Adam10 regulates endothelial permeability and t-cell transmigration by proteolysis of vascular endothelial cadherin. *Circ. Res.* **2008**, *102*, 1192–1201. [CrossRef]
90. Hattori, M.; Osterfield, M.; Flanagan, J.G. Regulated cleavage of a contact-mediated axon repellent. *Science* **2000**, *289*, 1360–1365. [CrossRef]
91. Pan, D.; Rubin, G.M. Kuzbanian controls proteolytic processing of notch and mediates lateral inhibition during drosophila and vertebrate neurogenesis. *Cell* **1997**, *90*, 271–280. [CrossRef]
92. Reiss, K.; Maretzky, T.; Ludwig, A.; Tousseyn, T.; de Strooper, B.; Hartmann, D.; Saftig, P. Adam10 cleavage of n-Cadherin and regulation of cell-cell adhesion and beta-catenin nuclear signalling. *EMBO J.* **2005**, *24*, 742–752. [CrossRef] [PubMed]
93. Inoshima, I.; Inoshima, N.; Wilke, G.A.; Powers, M.E.; Frank, K.M.; Wang, Y.; Bubeck Wardenburg, J. A staphylococcus aureus pore-forming toxin subverts the activity of adam10 to cause lethal infection in mice. *Nat. Med.* **2011**, *17*, 1310–1314. [CrossRef] [PubMed]
94. Sanderson, M.P.; Erickson, S.N.; Gough, P.J.; Garton, K.J.; Wille, P.T.; Raines, E.W.; Dunbar, A.J.; Dempsey, P.J. Adam10 mediates ectodomain shedding of the betacellulin precursor activated by p-aminophenylmercuric acetate and extracellular calcium influx. *J. Biol. Chem.* **2005**, *280*, 1826–1837. [CrossRef] [PubMed]
95. Woodin, A.M. Fractionation of a leucocidin from staphylococcus aureus. *Bioch. J.* **1959**, *73*, 225–237. [CrossRef] [PubMed]
96. Miles, G.; Movileanu, L.; Bayley, H. Subunit composition of a bicomponent toxin: Staphyloccocal leukocidin forms an octameric transmembrane pore. *Protein Sci.* **2002**, *11*, 894–902. [CrossRef]
97. DuMont, A.L.; Yoong, P.; Liu, X.; Day, C.J.; Chumble, N.M.; James, D.B.; Alonzo, F.; Bode, N.J.; Lacy, D.B.; Jennings, M.P.; et al. Identification of a crucial residue required for staphylococcus aureus lukab cytotoxicity and receptor recognition. *Infect. Immun.* **2014**, *82*, 1268–1276. [CrossRef]
98. Ventura, C.L.; Malachowa, N.; Hammer, C.H.; Nardone, G.A.; Robinson, M.A.; Kobayashi, S.D.; DeLeo, F.R. Identification of a novel staphylococcus aureus two-component leucotoxin using cell surface proteomics. *PLoS ONE* **2010**, *5*, e11634. [CrossRef]
99. Spaan, A.N.; Schippers, A.; de Haas, C.J.; van Hooijdonk, D.D.; Badiou, C.; Contamin, H.; Vandenesch, F.; Lina, G.; Gerard, N.P.; Gerard, C.; et al. Differential interaction of the staphylococcal toxins panton-valentine leukocidin and gamma-hemolysin cb with human c5a receptors. *J. Immunol.* **2015**, *195*, 1034–1043. [CrossRef]
100. Koop, G.; Vrieling, M.; Storisteaneu, D.M.; Lok, L.S.; Monie, T.; van Wigcheren, G.; Raisen, C.; Ba, X.; Gleadall, N.; Hadjirin, N.; et al. Identification of lukpq, a novel, equid-Adapted leukocidin of staphylococcus aureus. Sci. Rep. 2017, 7, 40660. [CrossRef] [PubMed]

101. Rainard, P.; Corrales, J.C.; Barrio, M.B.; Cochard, T.; Poutrel, B. Leucotoxic activities of staphylococcus aureus strains isolated from cows, ewes, and goats with mastitis: Importance of lukM/lukF'-PV leukotoxin. Clin. Diagn. Lab. Immunol. 2003, 10, 272–277. [CrossRef] [PubMed]

102. Holzinger, D.; Gieldon, L.; Mysore, V.; Pippe, N.; Taxman, D.J.; Duncan, J.A.; Broglie, P.M.; Marketon, K.; Austermann, J.; Vogl, T.; et al. Staphylococcus aureus panton-Valentine leukocidin induces an inflammatory response in human phagocytes via the nlrp3 inflammasome. J. Leukoc. Biol. 2012, 92, 1069–1081. [CrossRef] [PubMed]

103. Mariathasan, S.; Weiss, D.S.; McBride, J.; O'Rourke, K.; Roose-Girma, M.; Lee, W.P.; Weinrauch, Y.; Monack, D.M.; Dixit, V.M. Cryopyrin activates the inflammasome in response to toxins and ATP. Nature 2006, 440, 228–232. [CrossRef] [PubMed]

104. Melehani, J.H.; James, D.B.; DuMont, A.L.; Torres, V.J.; Duncan, J.A. Staphylococcus aureus leukocidin a/b (lukab) kills human monocytes via host nlrp3 and asp when extracellular, but not intracellular. PLoS Pathog. 2015, 11, e1004970. [CrossRef] [PubMed]

105. Munoz-Planillo, R.; Franchi, L.; Miller, L.S.; Nunez, G. A critical role for hemolysins and bacterial lipoproteins in staphylococcus aureus-Induced activation of the Nlrp3 inflammasome. J. Immunol. 2009, 183, 3942–3948. [CrossRef] [PubMed]

106. Perret, M.; Badiou, C.; Lina, G.; Burbaud, S.; Benito, Y.; Bes, M.; Cottin, V.; Couzon, F.; Juruj, C.; Dauwalder, O.; et al. Cross-Talk between staphylococcus aureus leukocidins-Intoxicated macrophages and lung epithelial cells triggers chemokine secretion in an inflammasome-dependent manner. Cell Microbiol. 2012, 14, 1019–1036. [CrossRef]

107. Graves, S.F.; Kobayashi, S.D.; Braughton, K.R.; Whitney, A.R.; Sturdevant, D.E.; Rasmussen, D.L.; Kirpotina, L.N.; Quinn, M.T.; DeLeo, F.R. Sublytic concentrations of staphylococcus aureus panton-Valentine leukocidin alters human p5a receptors. Cell Host Microbe 2013, 13, 584–594.

108. Spaan, A.N.; Henry, T.; van Rooijen, W.J.M.; Perret, M.; Badiou, C.; Aerts, P.C.; Kemmink, J.; de Haas, C.J.C.; van Kessel, K.P.M.; Vandenesch, F.; et al. The staphylococcal toxin panton-Valentine leukocidin targets human C5a receptors. Cell Host Microbe 2013, 13, 584–594.

109. Malachowa, N.; Kobayashi, S.D.; Freedman, B.; Dorward, D.W.; DeLeo, F.R. Staphylococcus aureus leukotoxin gh promotes formation of neutrophil extracellular traps. J. Immunol. 2013, 191, 6022–6029.

110. Kaneko, J.; Kimura, T.; Kawakami, Y.; Tomita, T.; Kamiyo, Y. Panton-Valentine leukocidin genes in a phage-like particle isolated from mitomycin c-Treated staphylococcus aureus v8 (atcc 49775). Biosci. Biotechnol. Biochem. 1997, 61, 1960–1962. [CrossRef]

111. Naimi, T.S.; LeDell, K.H.; Como-Sabetti, K.; Borchardt, S.M.; Boxrud, D.J.; Etienne, J.; Johnson, S.K.; Vandenesch, F.; Fridkin, S.; O’Boyle, C.; et al. Comparison of community-and health care-Associated methicillin-resistant staphylococcus aureus infection. JAMA 2003, 290, 2976–2984. [CrossRef] [PubMed]

112. Vandenesch, F.; Naimi, T.; Enright, M.C.; Lina, G.; Nimmo, G.R.; Heffernan, H.; Liassine, N.; Bes, M.; Greenland, T.; Reverdy, M.E.; et al. Community-Acquired methicillin-Resistant staphylococcus aureus carrying panton-valentine leukocidin genes: Worldwide emergence. Emerg. Infect. Dis. 2003, 9, 978–984. [CrossRef] [PubMed]

113. Lina, G.; Piemont, Y.; Godail-Gamot, F.; Bes, M.; Peter, M.O.; Gauduchon, V.; Vandenesch, F.; Etienne, J. Involvement of panton-valentine leukocidin-Producing staphylococcus aureus in primary skin infections and pneumonia. Clin. Infect. Dis. 1999, 29, 1128–1132. [CrossRef] [PubMed]

114. Shallcross, L.J.; Fragaszy, E.; Johnson, A.M.; Hayward, A.C. The role of the panton-valentine leucocidin toxin in staphylococcal disease: A systematic review and meta-Analysis. Lancet Infect. Dis. 2013, 13, 43–54. [CrossRef]

115. Shukla, S.K.; Karow, M.E.; Brady, J.M.; Stemper, M.E.; Kislow, J.; Moore, N.; Wroblewski, K.; Chyou, P.H.; Warshauer, D.M.; Reed, K.D.; et al. Virulence genes and genotypic associations in nasal carriage, community-associated methicillin-Susceptible and methicillin-Resistant USA400 staphylococcus aureus isolates. J. Clin. Microbiol. 2010, 48, 3582–3592. [CrossRef] [PubMed]
116. Alonzo, F.; Torres, V.J. The bicomponent pore-forming leucocidins of staphylococcus aureus. *Microbiol. Mol. Biol. Rev.* **2014**, *78*, 199–230. [CrossRef] [PubMed]

117. Lipinska, U.; Hermans, K.; Meulemans, L.; Dumitrescu, O.; Badiou, C.; Duchateau, L.; Hasebrouck, F.; Etienne, J.; Lina, G. Panton-Valentine leukocidin does play a role in the early stage of staphylococcus aureus skin infections: A rabbit model. *PLoS ONE* **2011**, *6*, e22864. [CrossRef] [PubMed]

118. Kobayashi, S.D.; Malachowa, N.; Whitney, A.R.; Braughton, K.R.; Gardner, D.J.; Long, D.; Bubeck Wardenburg, J.; Schneewind, O.; Otto, M.; Deleo, F.R. Comparative analysis of usa300 virulence determinants in a rabbit model of skin and soft tissue infection. *J. Infect. Dis.* **2011**, *204*, 937–941. [CrossRef]

119. Tromp, A.T.; Van Gent, M.; Abrial, P.; Martin, A.; Jansen, J.P.; De Haas, C.J.C.; Van Kessel, K.P.M.; Baroel, B.W.; Kruse, E.; Bourdonnay, E.; et al. Human CD45 is an f-Component-Specific receptor for the staphylococcal toxin panton-valentine leukocidin. *Nat. Microbiol.* **2018**, *3*, 708–717. [CrossRef]

120. Hermiston, M.L.; Xu, Z.; Weiss, A. Cd45: A critical regulator of signaling thresholds in immune cells. *Annu. Rev. Immunol.* **2003**, *21*, 107–137. [CrossRef]

121. Dozois, A.; Thomsen, I.; Jimenez-Truque, N.; Soper, N.; Pearson, A.; Mohamed-Rambaran, P.; Dettorre, K.B.; Creek, C.B.; Wright, S.W. Prevalence and molecular characteristics of methicillin-Resistant staphylococcus aureus among skin and soft tissue infections in an emergency department in guyana. *Emerg. Med. J.* **2015**, *32*, 800–803. [CrossRef]

122. Dumont, A.L.; Nygaard, T.K.; Watkins, R.L.; Smith, A.; Kozhaya, L.; Kreiswirth, B.N.; Shopsin, B.; Unutmaz, D.; Voyich, J.M.; Torres, V.J. Characterization of a new cytotoxin that contributes to staphylococcus aureus pathogenesis. *Mol. Microbiol.* **2011**, *79*, 814–825. [CrossRef]

123. DuMont, A.L.; Yoong, P.; Day, C.J.; Alonzo, F.; McDonald, W.H.; Jennings, M.P.; Torres, V.J. Staphylococcus aureus lukab cytotoxin kills human neutrophils by targeting the cd11b subunit of the integrin mac-1. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 10794–10799. [CrossRef]

124. Alonzo, F.; Kozhaya, L.; Rawlings, S.A.; Reyes-Robles, T.; DuMont, A.L.; Myszka, D.G.; Landau, N.R.; Unutmaz, D.; Torres, V.J. Ccr5 is a receptor for staphylococcus aureus leukotoxin ed. *Nature* **2013**, *493*, 51–55. [CrossRef] [PubMed]

125. Reyes-Robles, T.; Alonzo, F.; Kozhaya, L.; Lacy, D.B.; Unutmaz, D.; Torres, V.J. Staphylococcus aureus leukotoxin ed targets the chemokine receptors cxcr1 and cxcr2 to kill leukocytes and promote infection. *Cell Host Microbe* **2013**, *14*, 453–459. [CrossRef] [PubMed]

126. Spaan, A.N.; Reyes-Robles, T.; Badiou, C.; Cochet, S.; Boguslawski, K.M.; Yoong, P.; Day, C.J.; de Haas, C.J.; van Kessel, K.P.; Vandenesch, F.; et al. Staphylococcus aureus targets the duffy antigen receptor for chemokines (darc) to lyse erythrocytes. *Cell Host Microbe* **2015**, *18*, 363–370. [CrossRef] [PubMed]

127. Prevost, G.; Cribier, B.; Couppie, P.; Petiau, P.; Supersac, G.; Finck-Barbancon, V.; Monteil, H.; Piemont, Y. Panton-Valentine leukocidin does play a role in the early stage of staphylococcus aureus infection: A rabbit model. *Infect. Immun.* **2011**, *79*, 5883–5888. [CrossRef] [PubMed]

128. Peacock, S.J.; Moore, C.E.; Justice, A.; Kantzanou, M.; Story, L.; Mackie, K.; O’Neill, G.; Day, N.P. Virulent combinations of adhesin and toxin genes in natural populations of staphylococcus aureus. *PLoS ONE* **2011**, *6*, e21079. [CrossRef]

129. Fackrell, H.B.; Wiseman, G.M. Properties of the gamma haemolysin of staphylococcus aureus ‘smith 5r’. *J. Gen. Microbiol.* **1976**, *92*, 1–10. [CrossRef]

130. Fackrell, H.B.; Wiseman, G.M. Production and purification of the gamma haemolysin of staphylococcus aureus ‘smith 5r’. *J. Gen. Microbiol.* **1976**, *92*, 4987–4996. [CrossRef]

131. Spaan, A.N.; Vrieling, M.; Wallet, P.; Badiou, C.; Reyes-Robles, T.; Ohneck, E.A.; Benito, Y.; de Haas, C.J.; Day, C.J.; Jennings, M.P.; et al. The staphylococcal toxins gamma-Haemolysin ab and cb differentially target phagocytes by employing specific chemokine receptors. *Nat. Commun.* **2014**, *5*, 5438. [CrossRef] [PubMed]

132. Mehlin, C.; Headley, C.M.; Klebanoff, S.J. An inflammatory polypeptide complex from staphylococcus epidermidis: Isolation and characterization. *J. Exp. Med.* **1999**, *189*, 907–918. [CrossRef] [PubMed]

133. Wang, R.; Braughton, K.R.; Kretschmer, D.; Bach, T.H.; Queck, S.Y.; Li, M.; Kennedy, A.D.; Dorward, D.W.; Klebanoff, S.J.; Peschel, A.; et al. Identification of novel cytolytic peptides as key virulence determinants for community-Associated mrsa. *Nat. Med.* **2007**, *13*, 1510–1514. [CrossRef] [PubMed]

134. Li, M.; Diep, B.A.; Villaruz, A.E.; Braughton, K.R.; Jiang, X.; DeLeo, F.R.; Chambers, H.F.; Lu, Y.; Otto, M. Evolution of virulence in epidemic community-Associated methicillin-resistant staphylococcus aureus. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 5883–5888. [CrossRef] [PubMed]
135. Peschel, A.; Otto, M. Phenol-Soluble modulins and staphylococcal infection. Nat. Rev. Microbiol. 2013, 11, 667–673. [CrossRef] [PubMed]

136. Brosnahan, A.J.; Mantz, M.J.; Squier, C.A.; Peterson, M.L.; Schlievert, P.M. Cytolysins augment superantigenic penetration of stratified mucosa. J. Immunol. 2009, 182, 2364–2373. [CrossRef] [PubMed]

137. Gillman, A.N.; Breshers, L.M.; Kistler, C.K.; Finnegan, P.M.; Torres, V.J.; Schlievert, P.M.; Peterson, M.L. Epidermal growth factor receptor signaling enhances the proinflammatory effects of staphylococcus aureus gamma-toxin on the mucosa. Toxins 2017, 9, 202. [CrossRef] [PubMed]

138. Elliott, S.D. A proteolytic enzyme produced by group a streptococci with special reference to its effect on the type-specific m antigen. J. Exp. Med. 1945, 81, 573–592. [CrossRef]

139. Yu, C.E.; Ferretti, J.J. Frequency of the erythrogenic toxin-B and toxin-C genes (speb and spec) among clinical isolates of group-a streptococci. Infect. Immun. 1991, 59, 211–215. [CrossRef]

140. Liu, T.Y.; Elliott, S.D. Streptococcal proteinase: The zymogen to enzyme transformation. J. Biol. Chem. 1965, 240, 1138–1142.

141. Nyberg, P.; Rasmussen, M.; Von Pawel-Rammingen, U.; Bjorck, L. Speb modulates fibronectin-Dependent internalization of streptococcus pyogenes by efficient proteolysis of cell-Wall-Anchored protein F1. Microbiology 2004, 150, 1559–1569. [CrossRef] [PubMed]

142. Raeder, R.; Woischnik, M.; Podbielski, A.; Boyle, M.D.P. A secreted streptococcal cysteine protease can cleave a surface-Expressed M1 protein and alter the immunoglobulin binding properties. Res. Microbiol. 1998, 149, 539–548. [CrossRef]

143. Wexler, D.E.; Chenoweth, D.E.; Cleary, P.P. Mechanism of action of the group-a streptococcal C5a inactivator. Proc. Natl. Acad. Sci. USA 1985, 82, 8144–8148. [CrossRef] [PubMed]
154. Allhorn, M.; Olsen, A.; Collin, M. Endos from streptococcus pyogenes is hydrolyzed by the cysteine proteinase speb and requires glutamic acid 235 and tryptophans for igg glycan-Hydrolyzing activity. *BMC Microbiol.* **2008**, *8*, 3. [CrossRef] [PubMed]

155. Aziz, R.K.; Pabst, M.J.; Jung, A.; Kansal, R.; Low, D.E.; Nizet, V.; Kotb, M. Invasive m1t1 group a streptococcus undergoes a phase-Shift in vivo to prevent proteolytic degradation of multiple virulence factors by speb. *Mol. Microbiol.* **2004**, *51*, 123–134. [CrossRef] [PubMed]

156. Cole, J.N.; McArthur, J.D.; McKay, F.C.; Sanderson-Smith, M.L.; Cork, A.J.; Ranson, M.; Rohde, M.; Itzek, A.; Sun, H.; Ginsburg, D.; et al. Trigger for group a streptococcal m1t1 invasive disease. *FASEB J.* **2006**, *20*, 1745–1747. [CrossRef] [PubMed]

157. Pinkney, M.; Kapur, V.; Smith, J.; Weller, U.; Palmer, M.; Glanville, M.; Messner, M.; Musser, J.M.; Bhakdi, S.; Kehoe, M.A. Different forms of streptolysin o produced by streptococcus pyogenes and by escherichia coli expressing recombinant toxin: Cleavage by streptococcal cysteine protease. *Infect. Immun.* **1995**, *63*, 2776–2779. [PubMed]

158. Collin, M.; Olsen, A. Effect of speb and endos from streptococcus pyogenes on human immunoglobulins. *Infect. Immun.* **2001**, *69*, 7187–7189. [CrossRef]

159. Collin, M.; Svensson, M.D.; Sjoholm, A.G.; Jensenius, J.C.; Sjobring, U.; Olsen, A. Endos and speb from streptococcus pyogenes inhibit immunoglobulin-Mediated opsonophagocytosis. *Infect. Immun.* **2002**, *70*, 6646–6651. [CrossRef]

160. Kuo, C.F.; Lin, Y.S.; Chuang, W.J.; Wu, J.J.; Tsao, N. Degradation of complement 3 by streptococcal pyrogenic exotoxin b inhibits complement activation and neutrophil opsonophagocytosis. *Infect. Immun.* **2008**, *76*, 1163–1169. [CrossRef]

161. Terao, Y.; Mori, Y.; Yamaguchi, M.; Shimizu, Y.; Ooe, K.; Hamada, S.; Kawabata, S. Group a streptococcal cysteine protease degrades c3 (c3b) and contributes to evasion of innate immunity. *J. Biol. Chem.* **2008**, *283*, 6253–6260. [CrossRef] [PubMed]

162. Egesten, A.; Olin, A.I.; Linge, H.M.; Yadav, M.; Morgelin, M.; Karlsson, A.; Collin, M. Speb of streptococcus pyogenes differentially modulates antibacterial and receptor activating properties of human chemokines. *PLoS ONE* **2009**, *4*, e4769. [CrossRef] [PubMed]

163. Kapur, V.; Majesky, M.W.; Li, L.L.; Black, R.A.; Musser, J.M. Cleavage of interleukin 1 beta (il-1 beta) precursor to produce active IL-1 beta by a conserved extracellular cysteine protease from streptococcus pyogenes. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 7676–7680. [CrossRef] [PubMed]

164. LaRock, C.N.; Todd, J.; LaRock, D.L.; Olson, J.; O’Donoghue, A.J.; Robertson, A.A.B.; Cooper, M.A.; Hoffman, H.M.; Nizet, V. IL-1beta is an innate immune sensor of microbial proteolysis. *Sci. Immunol.* **2016**, *1*, eaah3539. [CrossRef] [PubMed]

165. Chella Krishnan, K.; Mukundan, S.; Alagarsamy, J.; Hur, J.; Nookala, S.; Siemens, N.; Svensson, M.; Hyldegaard, O.; Norrby-Teglund, A.; Kotb, M. Genetic architecture of group a streptococcal necrotizing soft tissue infections in the mouse. *PLoS Pathog.* **2016**, *12*, e1005732. [CrossRef] [PubMed]

166. Matsuka, Y.V.; Pillai, S.; Gubba, S.; Musser, J.M.; Olmsted, S.B. Fibrinogen cleavage by the streptococcus pyogenes extracellular cysteine protease and generation of antibodies that inhibit enzyme proteolytic activity. *Infect. Immun.* **1999**, *67*, 4326–4333. [PubMed]

167. Svensson, M.D.; Sjobring, U.; Luo, F.; Bessen, D.E. Roles of the plasminogen activator streptokinase and the plasminogen-associated m protein in an experimental model for streptococcal impetigo. *Microbiology* **2002**, *148*, 3933–3945. [CrossRef] [PubMed]

168. Kapur, V.; Topouzis, S.; Majesky, M.W.; Li, L.L.; Black, R.A.; Musser, J.M. A conserved streptococcus pyogenes extracellular cysteine protease necrotizes soft tissue infections in the mouse. *PLoS Pathog.* **2016**, *12*, e1005732. [CrossRef] [PubMed]
Toxins 2019, 11, 332

171. Luca-Harari, B.; Darenberg, J.; Neal, S.; Siljander, T.; Strakova, L.; Tanna, A.; Creti, R.; Ekelund, K.; Koliou, M.; Tassios, P.T.; et al. Clinical and microbiological characteristics of severe streptococcus pyogenes disease in europe. J. Clin. Microbiol. 2009, 47, 1155–1165. [CrossRef] [PubMed]

172. Gubba, S.; Low, D.E.; Musser, J.M. Expression and characterization of group a streptococcus extracellular cysteine protease recombinant mutant proteins and documentation of seroconversion during human invasive disease episodes. Infect. Immun. 1998, 66, 765–770. [PubMed]

173. Kansal, R.G.; McGeer, A.; Low, D.E.; Norrby-Teglund, A.; Kotb, M. Inverse relation between disease severity and expression of the streptococcal cysteine protease, speb, among clonal m1t1 isolates recovered from invasive group a streptococcal infection cases. Infect. Immun. 2000, 68, 6362–6369. [CrossRef] [PubMed]

174. Holm, S.E.; Norrby, A.; Bergholm, A.M.; Norgren, M. Aspects of pathogenesis of serious group—a streptococcal infections in sweden, 1988–1989. J. Infect. Dis. 1992, 166, 31–37. [CrossRef] [PubMed]

175. Kuo, C.F.; Wu, J.J.; Lin, K.Y.; Tsai, P.J.; Lee, S.C.; Jin, Y.T.; Lei, H.Y.; Lin, Y.S. Role of streptococcal pyrogenic exotoxin b in the mouse model of group a streptococcal infection. Infect. Immun. 1998, 66, 3931–3935.

176. Lukomski, S.; Burns, E.H., Jr.; Wyde, P.R.; Podbielski, A.; Rurangirwa, J.; Moore-Poveda, D.K.; Musser, J.M. Genetic inactivation of an extracellular cysteine protease (speb) expressed by streptococcus pyogenes decreases resistance to phagocytosis and dissemination to organs. Infect. Immun. 1998, 66, 771–776.

177. Lukomski, S.; Montgomery, C.A.; Rurangirwa, J.; Geske, R.S.; Barrish, J.P.; Adams, G.J.; Musser, J.M. Extracellular cysteine protease produced by streptococcus pyogenes participates in the pathogenesis of invasive skin infection and dissemination in mice. Infect. Immun. 1999, 67, 1779–1788.

178. Lukomski, S.; Sreevatsan, S.; Amberg, C.; Reichardt, W.; Woischneck, M.; Podbielski, A.; Musser, J.M. Inactivation of streptococcus pyogenes extracellular cysteine protease significantly decreases mouse lethality of serotype m3 and m49 strains. J. Clin. Investig. 1997, 99, 2574–2580. [CrossRef]

179. Ashbaugh, C.D.; Warren, H.B.; Carey, V.J.; Wessels, M.R. Molecular analysis of the role of the group a streptococcal cysteine protease, hyaluronic acid capsule, and m protein in a murine model of human invasive soft-tissue infection. J. Clin. Investig. 1998, 102, 550–560. [CrossRef]

180. Ashbaugh, C.D.; Wessels, M.R. Absence of a cysteine protease effect on bacterial virulence in two murine models of human invasive group a streptococcal infection. Infect. Immun. 2001, 69, 6683–6688. [CrossRef]

181. Von Pawel-Rammingen, U.; Johansson, B.P.; Bjorck, L. Ides, a novel streptococcal cysteine proteinase with unique specificity for immunoglobulin g. EMBO J. 2002, 21, 1607–1615. [CrossRef] [PubMed]

182. Von Pawel-Rammingen, U.; Johansson, B.P.; Tapper, H.; Bjorck, L. Streptococcus pyogenes and phagocytic killing. Nat. Med. 2002, 8, 1044–1045; author reply 1045–1046. [CrossRef] [PubMed]

183. O’Connor, S.P.; Cleary, P.P. Localization of the streptococcal C5a peptidase to the surface of group a streptococci. Infect. Immun. 1986, 53, 432–434. [PubMed]

184. Cleary, P.P.; Prahbu, U.; Dale, J.B.; Wexler, D.E.; Handley, J. Streptococcal c5a peptidase is a highly specific endopeptidase. Infect. Immun. 1992, 60, 5219–5223. [PubMed]

185. Ji, Y.D.; Carlson, B.; Kondagunta, A.; Cleary, P.P. Intranasal immunization with C5a peptidase prevents nasopharyngeal colonization of mice by the group a streptococcus. Infect. Immun. 1997, 65, 2080–2087. [PubMed]

186. Lynskey, N.N.; Reglinski, M.; Calay, D.; Siggins, M.K.; Mason, J.C.; Botto, M.; Sriskandan, S. Multi-Functional mechanisms of immune evasion by the streptococcal complement inhibitor c5a peptidase. PLoS Pathog. 2017, 13, e1006493. [CrossRef] [PubMed]

187. Hidalgo-Grass, C.; Dan-Goor, M.; Maly, A.; Eran, Y.; Kwinn, L.A.; Nizet, V.; Ravins, M.; Jaffe, J.; Peyser, A.; Moses, A.E.; et al. Effect of a bacterial pheromone peptide on host chemokine degradation in group a streptococcal necrotising soft-Tissue infections. Lancet 2004, 363, 696–703. [CrossRef]

188. Hidalgo-Grass, C.; Mishalian, I.; Dan-Goor, M.; Belotserkovsky, I.; Eran, Y.; Nizet, V.; Peled, A.; Hanski, E. A streptococcal protease that degrades cxc chemokines and impairs bacterial clearance from infected tissues. EMBO J. 2006, 25, 4628–4637. [CrossRef]

189. Zinkernagel, A.S.; Timmer, A.M.; Pence, M.A.; Locke, J.B.; Buchanan, J.T.; Turner, C.E.; Mishalian, I.; Sriskandan, S.; Hanski, E.; Nizet, V. The il-8 protease spycep/scpc of group a streptococcus promotes resistance to neutrophil killing. Cell Host Microbe 2008, 4, 170–178. [CrossRef]
190. Sumby, P.; Zhang, S.; Whitney, A.R.; Falugi, F.; Grandi, G.; Graviss, E.A.; DeLeo, F.R.; Musser, J.M. A chemokine-degrading extracellular protease made by group a streptococcus alters pathogenesis by enhancing evasion of the innate immune response. *Infect. Immun.* 2008, 76, 978–985. [CrossRef]

191. Chiappini, N.; Seubert, A.; Telford, J.L.; Grandi, G.; Serruto, D.; Margarit, I.; Janulczyk, R. Streptococcus pyogenes spycep influences host-Pathogen interactions during infection in a murine air pouch model. *PLoS ONE* 2012, 7, e40411. [CrossRef] [PubMed]

192. Andreoni, F.; Ogawa, T.; Ogawa, M.; Madon, J.; Uchiyama, S.; Schuepbach, R.A.; Zinkernagel, A.S. The il-8 protease spycep is detrimental for group a streptococcus host-Cells interaction and biofilm formation. *Front. Microbiol.* 2014, 5, 339. [CrossRef] [PubMed]

193. Madden, J.C.; Ruiz, N.; Caparon, M. Cytolysin-mediated translocation (cmt): A functional equivalent of type iii secretion in gram-Positive bacteria. *Cell* 2001, 104, 143–152. [CrossRef]

194. Chandrasekaran, S.; Caparon, M.G. The streptococcus pyogenes nad(+)-glycohydrolase modulates epithelial cell parylation and hmgb1 release. *Cell Microbiol.* 2015, 17, 1376–1390. [CrossRef] [PubMed]

195. Velarde, J.J.; O’Séaghdha, M.; Baddal, B.; Bastiat-Sempe, B.; Wessels, M.R. Binding of nad(+)-glycohydrolase to streptolysin o stabilizes both toxins and promotes virulence of group a streptococcus. *mBio* 2017, 8, e01382-17. [CrossRef] [PubMed]

196. Coye, L.H.; Collins, C.M. Identification of spya, a novel adp-Ribosyltransferase of streptococcus pyogenes. *Mol. Microbiol.* 2004, 54, 89–98. [CrossRef] [PubMed]

197. Fox, J.S.; DeWald, M.; Moseley, S.L.; Collins, C.M.; Voyich, J.M. Spya, a C3-like adp-Ribosyltransferase, contributes to virulence in a mouse subcutaneous model of streptococcus pyogenes infection. *Infect. Immun.* 2011, 79, 2404–2411. [CrossRef]

198. Korotkova, N.; Hoff, J.S.; Becker, D.M.; Quinn, J.K.H.; Icenogle, L.M.; Moseley, S.L. Spya is a membrane-bound adp-ribosyltransferase of streptococcus pyogenes which modifies a streptococcal peptide, spyb. *Mol. Microbiol.* 2012, 83, 936–952. [CrossRef]

199. Lin, A.E.; Beasley, F.C.; Keller, N.; Hollands, A.; Urbano, R.; Troemel, E.R.; Hoffman, H.M.; Nizet, V. A group a streptococcus adp-ribosyltransferase toxin stimulates a protective interleukin 1beta-Dependent macrophage immune response. *mBio* 2015, 6, e00133. [CrossRef]

200. Hynes, W.; Sloan, M. Secreted extracellular virulence factors. In *Streptococcus Pyogenes: Basic Biology to Clinical Manifestations*; Ferretti, J.J., Stevens, D.L., Fischetti, V.A., Eds.; University of Oklahoma Health Sciences Center: Oklahoma, OK, USA, 2016.

201. Kalia, A.; Bessen, D.E. Natural selection and evolution of streptococcal virulence genes involved in tissue-specific adaptations. *J. Bacteriol.* 2004, 186, 110–121. [CrossRef]

202. Chandrachas, V.; Glinton, K.; Liang, Z.; Donahue, D.L.; Ploplis, V.A.; Castellino, F.J. Direct host plasminogen binding to bacterial surface m-protein in pattern d strains of streptococcus pyogenes is required for activation by its natural coinherited SK2b protein. *J. Biol. Chem.* 2015, 290, 18833–18842. [CrossRef] [PubMed]

203. Siemens, N.; Palenge, N.; Otto, J.; Fiedler, T.; Kreikemeyer, B. Streptococcus pyogenes M49 plasminogen/plasmin binding facilitates keratinocyte invasion via integrin-integrin-linked kinase (ILK) pathways and protects from macrophage killing. *J. Biol. Chem.* 2011, 286, 21612–21622. [CrossRef] [PubMed]

204. Boxrud, P.D.; Bock, P.E. Coupling of conformational and proteolytic activity in the kinetic mechanism of plasminogen activation by streptokinase. *J. Biol. Chem.* 2004, 279, 36642–36649. [CrossRef] [PubMed]

205. Boxrud, P.D.; Verhamme, I.M.; Bock, P.E. Resolution of conformational activation in the kinetic mechanism of plasminogen activation by streptokinase. *J. Biol. Chem.* 2004, 279, 36633–36641. [CrossRef] [PubMed]

206. Khil, J.; Im, M.; Heath, A.; Ringdahl, U.; Mundada, L.; Cary Engleberg, N.; Fay, W.P. Plasminogen enhances virulence of group a streptococci by streptokinase-dependent and streptokinase-independent mechanisms. *J. Infect. Dis.* 2003, 188, 497–505. [CrossRef] [PubMed]

207. Sun, H.; Ringdahl, U.; Homeister, J.W.; Fay, W.P.; Engleberg, N.C.; Yang, A.Y.; Rozek, L.S.; Wang, X.; Sjobring, U.; Ginsburg, D. Plasminogen is a critical host pathogenicity factor for group a streptococcal infection. *Science* 2004, 305, 1283–1286. [CrossRef]

208. Shaw, L.; Golonka, E.; Potempa, J.; Foster, S.J. The role and regulation of the extracellular proteases of staphylococcus aureus. *Microbiology* 2004, 150, 217–228. [CrossRef] [PubMed]

209. Drappeau, G.R. Role of metalloprotease in activation of the precursor of staphylococcal protease. *J. Bacteriol.* 1978, 136, 607–613. [PubMed]
210. Ohbayashi, T.; Irie, A.; Murakami, Y.; Nowak, M.; Potempa, J.; Nishimura, Y.; Shinozawa, M.; Imamura, T. Degradation of fibrinogen and collagen by staphopains, cysteine proteinases released from staphylococcus aureus. *Microbiology* 2011, 157, 786–792. [CrossRef]

211. Potempa, J.; Dubin, A.; Korzus, G.; Travis, J. Degradation of elastin by a cysteine proteinase from staphylococcus aureus. *J. Biol. Chem.* 1988, 263, 2664–2667.

212. Kuhn, M.L.; Prachi, P.; Minasov, G.; Shuvalova, L.; Ruan, J.; Dubrovska, I.; Winsor, J.; Giraldi, M.; Biagini, M.; Prokesova, L.; Potuznikova, B.; Potempa, J.; Zikan, J.; Radl, J.; Hachova, L.; Baran, K.; Porwit-Bobr, Z.; John, C. Smagur, J.; Guzik, K.; Bzowska, M.; Kuzak, M.; Zarebski, M.; Kantyka, T.; Walski, M.; Gajkowska, B.; Laarman, A.J.; Mijnheer, G.; Mootz, J.M.; van Rooijen, W.J.; Ruyken, M.; Malone, C.L.; Heezius, E.C.; Ward, R.; Ohbayashi, T.; Irie, A.; Murakami, Y.; Nowak, M.; Potempa, J.; Nishimura, Y.; Shinohara, M.; Imamura, T. Staphylococcal exfoliative proteinases from staphylococcus aureus. *Antimicrob. Agents Chemother.* 2017, 61, 259–265. [CrossRef] [PubMed]

213. Banbula, A.; Potempa, J.; Travis, J.; Fernandez-Catalan, C.; Mann, K.; Huber, R.; Bode, W.; Medrano, F. Molecular characterization of a novel staphylococcus aureus serine protease operon. *Front. Cell Infect. Microbiol.* 2017, 7, 166. [CrossRef] [PubMed]

214. Potempa, J.; Watorek, W.; Travis, J. The inactivation of human plasma alpha 1-Proteinase inhibitor by staphylococcus aureus. *Infect. Immun.* 2001, 69, 1521–1527. [CrossRef] [PubMed]

215. Piecuch, M.; Amagai, M.; Yamaguchi, T.; Hanakawa, Y.; Nishifuji, K.; Sugai, M.; Stanley, J.R. Staphylococcal exfoliative toxin a and toxin b genes from staphylococcus aureus. *J. Bacteriol.* 1987, 169, 3904–3909. [CrossRef]

216. Popowicz, G.M.; Dubin, G.; Stc-Niemczyk, J.; Czarny, A.; Dubin, A.; Potempa, J.; Holak, T.A. Functional and structural characterization of spl proteinases from staphylococcus aureus. *J. Mol. Biol.* 2006, 358, 270–279. [CrossRef] [PubMed]

217. Stentzel, S.; Teufelberger, A.; Nordengrun, M.; Kolata, J.; Schmidt, F.; van Crombruggen, K.; Michalik, S.; Kumpfmuller, J.; Tischer, S.; Schweder, T.; et al. Staphylococcal serine protease-like proteins are pacemakers of allergic airway reactions to staphylococcus aureus. *J. Allergy Clin. Immunol.* 2017, 139, 492–500.e8. [CrossRef] [PubMed]

218. Banbula, A.; Potempa, J.; Travis, J.; Fernandez-Catalan, C.; Mann, K.; Huber, R.; Bode, W.; Medrano, F. Amino-Acid sequence and three-dimensional structure of the staphylococcus aureus metalloproteinase at 1.72 a resolution. *Structure* 1998, 6, 1185–1193. [CrossRef]

219. Potempa, J.; Watorek, W.; Travis, J. The inactivation of human plasma alpha 1-Proteinase inhibitor by proteinases from staphylococcus aureus. *J. Biol. Chem.* 1986, 261, 14330–14334.

220. Burlak, C.; Hammer, C.H.; Robinson, M.A.; Whitney, A.R.; McGavin, M.J.; Kreiswirth, B.N.; Deleo, F.R. Global analysis of community-Associated methicillin-Resistant staphylococcus aureus exoproteins reveals molecules produced in vitro and during infection. *Cell Microbiol.* 2007, 9, 1172–1190. [CrossRef]

221. Sieprowska-Lupa, M.; Mydel, P.; Krawczyk, K.; Wojcik, K.; Puklo, M.; Lupa, B.; Suder, P.; Silberring, J.; Reed, M.; Pohl, J.; et al. Degradation of human antimicrobial peptide LL-37 by staphylococcus aureus-Derived proteinases. *Antimicrob. Agents Chemother.* 2004, 48, 4673–4679. [CrossRef] [PubMed]
228. Laarman, A.J.; Ruyken, M.; Malone, C.L.; van Strijp, J.A.; Horswill, A.R.; Rooijakkers, S.H. Staphylococcus aureus metalloprotease aureolysin cleaves complement C3 to mediate immune evasion. *J. Immunol.* 2011, 186, 6445–6453. [CrossRef]

229. Peetermans, M.; Vanassche, T.; Liesenborghs, L.; Lijnen, R.H.; Verhamme, P. Bacterial pathogens activate plasminogen to breach tissue barriers and escape from innate immunity. *Crit. Rev. Microbiol.* 2016, 42, 866–882. [CrossRef]

230. Bokarewa, M.I.; Jin, T.; Tarkowski, A. Staphylococcus aureus: Staphylokinase. *Int. J. Biochem. Cell Biol.* 2006, 38, 504–509. [CrossRef]

231. Braff, M.H.; Jones, A.L.; Skerrett, S.J.; Rubens, C.E. Staphylococcus aureus exploits cathelicidin antimicrobial peptides produced during early pneumonia to promote staphylokinase-dependent fibrinolysis. *J. Infect. Dis.* 2007, 195, 1365–1372. [CrossRef]

232. Jin, T.; Bokarewa, M.; Foster, T.; Mitchell, J.; Higgins, J.; Tarkowski, A. Staphylococcus aureus resists human defensins by production of staphylokinase, a novel bacterial evasion mechanism. *J. Immunol.* 2004, 172, 1169–1176. [CrossRef] [PubMed]

233. Pietrocola, G.; Nobile, G.; Gianotti, V.; Zapotoczna, M.; Foster, T.J.; Geoghegan, J.A.; Speziale, P. Molecular interactions of human plasminogen with fibronectin-binding protein b (fnbpb), a fibrinogen/fibronectin-Binding protein from staphylococcus aureus. *J. Biol. Chem.* 2016, 291, 18148–18162. [CrossRef] [PubMed]

234. Rooijakkers, S.H.; van Wamel, W.J.; Ruyken, M.; van Kessel, K.P.; van Strijp, J.A. Anti-Opsonic properties of staphylokinase. *Microbes Infect.* 2005, 7, 476–484. [CrossRef] [PubMed]

235. Churchward, G. The two faces of janus: Virulence gene regulation by covR/S in group a streptococci. *Mol. Microbiol.* 2007, 64, 34–41. [CrossRef] [PubMed]

236. Jenul, C.; Horswill, A.R. Regulation of staphylococcus aureus virulence. *Microbiol. Spectr.* 2018, 6. [CrossRef] [PubMed]

237. Bronner, S.; Stoessel, P.; Gravet, A.; Monteil, H.; Prevost, G. Variable expressions of staphylococcus aureus bicomponent leucotoxins semiquantified by competitive reverse transcription-PCR. *Appl. Environ. Microbiol.* 2000, 66, 3931–3938. [CrossRef] [PubMed]

238. Malachowa, N.; Whitney, A.R.; Kobayashi, S.D.; Sturdevant, D.E.; Kennedy, A.D.; Braughton, K.R.; Shabb, D.W.; Diep, B.A.; Chambers, H.F.; Otto, M.; et al. Global changes in staphylococcus aureus gene expression in human blood. *PLoS ONE* 2011, 6, e18617. [CrossRef] [PubMed]

239. Loughman, J.A.; Fritz, S.A.; Storch, G.A.; Hunstad, D.A. Virulence gene expression in human community-Acquired staphylococcus aureus infection. *J. Infect. Dis.* 2009, 199, 294–301. [CrossRef] [PubMed]

240. Bronesky, D.; Wu, Z.; Marzi, S.; Walter, P.; Geissmann, T.; Moreau, K.; Vandenesch, F.; Caldelari, I.; Rompy, B. Staphylococcus aureus mraI and its regulon link quorum sensing, stress responses, metabolic adaptation, and regulation of virulence gene expression. *Annu. Rev. Microbiol.* 2016, 70, 299–316. [CrossRef]

241. Le, K.Y.; Otto, M. Quorum-sensing regulation in staphylococci—An overview. *Front. Microbiol.* 2015, 6, 1174. [CrossRef] [PubMed]

242. Novick, R.P.; Ross, H.F.; Projan, S.J.; Kornblum, J.; Kreiswirth, B.; Moghazeh, S. Synthesis of staphylococcal virulence factors is controlled by a regulatory rna molecule. *EMBO J.* 1993, 12, 3967–3975. [CrossRef] [PubMed]

243. Fowler, V.G., Jr.; Sakoulas, G.; McIntyre, L.M.; Meka, V.G.; Arbet, R.D.; Cabell, C.H.; Stryjewski, M.E.; Eliopoulos, G.M.; Reller, L.B.; Corey, G.R.; et al. Persistent bacteremia due to methicillin-Resistant staphylococcus aureus infection is associated with agr dysfunction and low-level in vitro resistance to thrombin-Induced platelet microbicidal protein. *J. Infect. Dis.* 2004, 190, 1140–1149. [CrossRef] [PubMed]

244. Shopsin, B.; Drlica-Wagner, A.; Mathema, B.; Adhikari, R.P.; Kreiswirth, B.N.; Novick, R.P. Prevalence of agr dysfunction among colonizing staphylococcus aureus strains. *J. Infect. Dis.* 2008, 198, 1171–1174. [CrossRef] [PubMed]

245. Mairpady Shambat, S.; Siemens, N.; Monk, I.R.; Mohan, D.B.; Mukundan, S.; Krishnan, K.C.; Prabhakara, S.; Snall, J.; Kearns, A.; Vandenesch, F.; et al. A point mutation in agrc determines cytotoxic or colonizing properties associated with phenotypic variants of st22 mrsa strains. *Sci. Rep.* 2016, 6, 31360. [CrossRef] [PubMed]
Toxins 2019, 11, 332

246. Cheung, G.Y.; Wang, R.; Khan, B.A.; Sturdevant, D.E.; Otto, M. Role of the accessory gene regulator agr in community-Associated methicillin-Resistant staphylococcus aureus pathogenesis. Infect. Immun. 2011, 79, 1927–1935. [CrossRef]

247. Liu, Q.; Yeo, W.S.; Bae, T. The saeR-two Component system of staphylococcus aureus. Genes 2016, 7, 81. [CrossRef] [PubMed]

248. Giraudo, A.T.; Calzolari, A.; Cataldi, A.A.; Bogri, C.; Nagel, R. The sae locus of staphylococcus aureus encodes a two-component regulatory system. FEMS Microbiol. Lett 1999, 177, 15–22. [CrossRef] [PubMed]

249. Voyich, J.M.; Vuong, C.; DeWald, M.; Nygaard, T.K.; Kocianova, S.; Griffith, S.; Jones, J.; Iverson, C.; Sturdevant, D.E.; Braughton, K.R.; et al. The saeRS gene regulatory system is essential for innate immune evasion by staphylococcus aureus. J. Infect. Dis. 2009, 199, 1698–1706. [CrossRef]

250. Geiger, T.; Goerke, C.; Mainiero, M.; Kraus, D.; Wolz, C. The virulence regulator sae of staphylococcus aureus: Promoter activities and response to phagocytosis-related signals. J. Bacteriol. 2008, 190, 3419–3428. [CrossRef]

251. Novick, R.P.; Jiang, D. The staphylococcal saeRs system coordinates environmental signals with agr quorum sensing. Microbiology 2003, 149, 2709–2717. [CrossRef]

252. Baroja, M.L.; Herfst, C.A.; Kasper, K.J.; Xu, S.X.; Gillett, D.A.; Li, J.; Reid, G.; McCormick, J.K. The sae two-Component system is a direct and dominant transcriptional activator of toxic shock syndrome toxin 1 in staphylococcus aureus. J. Bacteriol. 2016, 198, 2732–2742. [CrossRef] [PubMed]

253. Zurek, O.W.; Nygaard, T.K.; Watkins, R.L.; Pallister, K.B.; Torres, V.J.; Horswill, A.R.; Voyich, J.M. The role of innate immunity in promoting saeRS-Mediated virulence in staphylococcus aureus. J. Innate Immun. 2014, 6, 21–30. [CrossRef] [PubMed]

254. Engleberg, N.C.; Heath, A.; Miller, A.; Rivera, C.; DiRita, V.J. Spontaneous mutations in the crrs two-Component regulatory system of streptococcus pyogenes result in enhanced virulence in a murine model of skin and soft tissue infection. J. Infect. Dis. 2001, 183, 1043–1054. [CrossRef] [PubMed]

255. Madsen, M.B.; Hjortrup, P.B.; Hansen, M.B.; Lange, T.; Norby-Seglund, A.; Hyldegaard, O.; Perner, A. Immunoglobulin g for patients with necrotising soft tissue infection (instinct): A randomised, blinded, placebo-controlled trial. Intensive Care Med. 2017, 43, 1585–1593. [CrossRef] [PubMed]

256. Polzik, P.; Johansson, P.I.; Hyldegaard, O. How biomarkers reflect the prognosis and treatment of necrotising soft tissue infections and the effects of hyperbaric oxygen therapy: The protocol of the prospective cohort protreat study conducted at a tertiary hospital in copenhagen, denmark. BMJ Open 2017, 7, e017805. [PubMed]

257. Stevens, D.L.; Bisno, A.L.; Chambers, H.F.; Dellinger, E.P.; Goldstein, E.J.; Gorbach, S.L.; Hirschmann, J.V.; Kaplan, S.L.; Montoya, J.G.; Wade, J.C.; et al. Practice guidelines for the diagnosis and management of skin and soft tissue infections: 2014 update by the infectious diseases society of america. Clin. Infect. Dis. 2014, 59, e10–e52. [CrossRef] [PubMed]

258. Freischlag, J.A.; Ajalat, G.; Busuttil, R.W. Treatment of necrotizing soft tissue infections. The need for a new approach. Am. J. Surg. 1985, 149, 751–755. [CrossRef]

259. McHenry, C.R.; Piotrowski, J.J.; Petrinic, D.; Malangoni, M.A. Determinants of mortality for necrotizing fasciitis can improve survival: An observational intensive care unit cohort study. Ann. Surg. 2013, 386, 365–370. [CrossRef]

260. Hadeed, G.J.; Smith, J.; O’Keefe, T.; Kulvatunyou, N.; Wynne, J.L.; Joseph, B.; Friese, R.S.; Wachtel, T.L.; Rhee, P.M.; El-Menyar, A.; et al. Early surgical intervention and its impact on patients presenting with necrotizing soft tissue infections: A single academic center experience. J. Emerg. Trauma Shock 2016, 9, 22–27.

261. Stevens, D.L.; Gibbons, A.E.; Bergstrom, R.; Winn, V. The eagle effect revisited: Efficacy of clindamycin, erythromycin, and penicillin in the treatment of streptococcal myositis. J. Infect. Dis. 1988, 158, 23–28. [PubMed]

262. Sriskandan, S.; McKee, A.; Hall, L.; Cohen, J. Comparative effects of clindamycin and ampicillin on superantigenic activity of streptococcus pyogenes. J. Antimicrob. Chemother. 1997, 40, 275–277. [CrossRef] [PubMed]

263. Linner, A.; Darenberg, J.; Sjolin, J.; Henriques-Normark, B.; Norrby-Teglund, A. Clinical efficacy of polyspecific intravenous immunoglobulin therapy in patients with streptococcal toxic shock syndrome: A comparative observational study. Clin. Infect. Dis. 2014, 59, 851–857. [CrossRef] [PubMed]
265. Andreoni, F.; Zurcher, C.; Tarnutzer, A.; Schilcher, K.; Neff, A.; Keller, N.; Marques Maggio, E.; Poyart, C.; Schupbach, R.A.; Zinkernagel, A.S. Clindamycin affects group a streptococcus virulence factors and improves clinical outcome. J. Infect. Dis. 2017, 215, 269–277. [PubMed]

266. Coyle, E.A.; Cha, R.; Rybak, M.J. Influences of linezolid, penicillin, and clindamycin, alone and in combination, on streptococcal pyrogenic exotoxin a release. Antimicrob. Agents Chemother. 2003, 47, 1752–1755. [CrossRef] [PubMed]

267. DeMuri, G.P.; Sterkel, A.K.; Kubica, P.A.; Duster, M.N.; Reed, K.D.; Wald, E.R. Macrolide and clindamycin resistance in group a streptococci isolated from children with pharyngitis. Pediatr. Infect. Dis. J. 2017, 36, 342–344. [CrossRef]

268. Eckmann, C.; Dryden, M. Treatment of complicated skin and soft-Tissue infections caused by resistant bacteria: Value of linezolid, tigecycline, daptomycin and vancomycin. Eur. J. Med. Res. 2010, 15, 554–563.

269. Dryden, M.S. Complicated skin and soft tissue infection. J. Antimicrob. Chemother. 2010, 65, iii35–iii44. [CrossRef]

270. Bounthavong, M.; Hsu, D.I. Efficacy and safety of linezolid in methicillin-Resistant staphylococcus aureus (mrsa) complicated skin and soft tissue infection (cssti): A meta-Analysis. Curr. Med. Res. Opin. 2010, 26, 407–421. [CrossRef]

271. Itani, K.M.; Dryden, M.S.; Bhattacharyya, H.; Kunkel, M.J.; Baruch, A.M.; Weigelt, J.A. Efficacy and safety of linezolid versus vancomycin for the treatment of complicated skin and soft-tissue infections proven to be caused by methicillin-Resistant staphylococcus aureus. Am. J. Surg. 2010, 199, 804–816. [CrossRef]

272. Stevens, D.L.; Herr, D.; Lampiris, H.; Hunt, J.L.; Batts, D.H.; Hafkin, B. Linezolid versus vancomycin for the treatment of methicillin-Resistant staphylococcus aureus infections. Clin. Infect. Dis. 2002, 34, 1481–1490. [CrossRef] [PubMed]

273. Miller, W.R.; Bayer, A.S.; Arias, C.A. Mechanism of action and resistance to daptomycin in staphylococcus aureus and enterococci. Cold Spring Harb. Perspect. Med. 2016, 6, a026997. [CrossRef] [PubMed]

274. Mairpady Shambat, S.; Haggar, A.; Vandenesch, F.; Lina, G.; van Wamel, W.J.; Arakere, G.; Svensson, M.; Norrby-Teglund, A. Levels of alpha-Toxin correlate with distinct phenotypic response profiles of blood mononuclear cells and with agr background of community-Associated staphylococcus aureus isolates. PLoS ONE 2014, 9, e106107. [CrossRef] [PubMed]

275. Norrby-Teglund, A.; Ihendyane, N.; Kansal, R.; Basma, H.; Kotb, M.; Andersson, J.; Hammarstrom, L. Relative neutralizing activity in polyspecific igm, iga, and igg preparations against group a streptococcal superantigens. Clin. Infect. Dis. 2000, 31, 1175–1182. [CrossRef] [PubMed]

276. Norrby-Teglund, A.; Kaul, R.; Low, D.E.; McGeer, A.; Newton, D.W.; Andersson, J.; Andersson, U.; Kotb, M. Plasma from patients with severe invasive group a streptococcal infections treated with normal polyspecific igg inhibits streptococcal superantigen-induced t cell proliferation and cytokine production. J. Immunol. 1996, 156, 3057–3064. [PubMed]

277. Norrby-Teglund, A.; Low, D.E.; McGeer, A.; Kotb, M. Superantigenic activity produced by group a streptococcal isolates is neutralized by plasma from IVIG-Treated streptococcal toxic shock syndrome patients. Adv. Exp. Med. Biol. 1997, 418, 563–566. [PubMed]

278. Tarnutzer, A.; Andreoni, F.; Keller, N.; Zurcher, C.; Norrby-Teglund, A.; Schupbach, R.A.; Zinkernagel, A.S. Human polyspecific immunoglobulin attenuates group a streptococcal virulence factor activity and reduces disease severity in a murine necrotizing fasciitis model. Clin. Microbiol. Infect. 2018, 25, 512.e7–512.e13. [CrossRef] [PubMed]

279. Norrby-Teglund, A.; Muller, M.P.; McGeer, A.; Gan, B.S.; Guru, V.; Bohnen, J.; Thulin, P.; Low, D.E. Successful management of severe group a streptococcal soft tissue infections using an aggressive medical regimen including intravenous polyspecific immunoglobulin together with a conservative surgical approach. scand. J. Infect. Dis. 2005, 37, 166–172. [CrossRef]

280. Parks, T.; Wilson, C.; Curtis, N.; Norrby-Teglund, A.; Sriskandan, S. Polyspecific intravenous immunoglobulin in clindamycin-treated patients with streptococcal toxic shock syndrome: A systematic review and meta-analysis. Clin. Infect. Dis. 2018, 67, 1434–1436. [CrossRef]

281. Shaw, J.J.; Psinosos, C.; Emhoff, T.A.; Shah, S.A.; Santry, H.P. Not just full of hot air: Hyperbaric oxygen therapy increases survival in cases of necrotizing soft tissue infections. Surg. Infect. 2014, 15, 328–335. [CrossRef]
282. Wang, C.; Schwatzberg, S.; Berliner, E.; Zarin, D.A.; Lau, J. Hyperbaric oxygen for treating wounds: A systematic review of the literature. *Arch. Surg.* **2003**, *138*, 272–279. [CrossRef] [PubMed]

283. Hansen, M.B.; Simonsen, U.; Garred, P.; Hyldegaard, O. Biomarkers of necrotising soft tissue infections: Aspects of the innate immune response and effects of hyperbaric oxygenation-the protocol of the prospective cohort bionec study. *BMJ Open* **2015**, *5*, e006995. [CrossRef] [PubMed]