Staphylococcus aureus-Derived Extracellular Vesicles and Atopic Dermatitis Pathophysiology

Subjects: Immunology

Contributor: Marina Passos Torrealba , Fabio Seiti Yamada Yoshikawa , Valeria Aoki , Maria Notomi Sato , Raquel Leão Orfali

Atopic dermatitis (AD) is a chronic and relapsing inflammatory cutaneous disease. The role of host defense and microbial virulence factors in *Staphylococcus aureus* skin colonization, infection, and inflammation perpetuation in AD remains an area of current research focus. Extracellular vesicles (EV) mediate cell-to-cell communication by transporting and delivering bioactive molecules, such as nucleic acids, proteins, and enzymes, to recipient cells. *Staphylococcus aureus* spontaneously secretes extracellular vesicles (SA-derived EVs), which spread throughout the skin layers. Research has shown that SA-derived EVs from AD patients can trigger cytokine secretion in keratinocytes, shape the recruitment of neutrophils and monocytes, and induce inflammatory AD-type lesions in mouse models, in addition to their role as exogenous worsening factors for the disease.

Staphylococcus aureus | extracellular vesicles | atopic dermatitis | pathogenesis | α-hemolysin

1. Introduction

Atopic dermatitis (AD) is a chronic and relapsing inflammatory skin disease. It shows worldwide occurrence, affecting both children and adults, whose prevalence is approximately 15–20% and 1–5%, respectively [1][2][3]. Although a common disease, the pathogenesis of AD is a complex network of skin barrier defects, imbalance in adaptive and innate immunity, and chronic skin colonization by *Staphylococcus aureus* (*S. aureus*) [4][5][6], all of which drive an inflammatory vicious cycle culminating in the main symptom: pruritus [7][8][9][10]. But among the variables associated with AD development, the interaction between the host defense system and the microbial virulence factors involved in *S. aureus* skin colonization, infection and the perpetuation of inflammation are gathering increased attention [11], mostly due to clinical evidence that, in AD flares, decreased bacterial diversity associated with increased *S. aureus* abundance has been described and related to disease severity [11][12][13][14].

The virulence factors of *S. aureus* include the secretion of numerous exotoxins, comprising an assembly of polypeptides capable of injuring the host cell plasma membrane, the called pore-forming toxins: α-hemolysin and the bi-component leukocidins γ-hemolysin, the Panton Valentine leukocidin, LukED, and LukGH/AB, β-hemolysin (neutral sphingomyelinase), and the phenol soluble modulins (small amphipathic peptides), as well as

staphylococcal enterotoxins (SEA to SEE, SEG to SEJ, SEL to SEQ and SER to SET), and 11 staphylococcal superantigen-like (SSL) toxins (SEIK to SEIQ, SEIU to SEIX) [15][16].

Almost all cell types release double-lipid membrane structures termed “extracellular vesicles (EVs)” into the extracellular lumen. EV production is a conserved biological phenomenon, considered a fundamental feature in all three domains of life: eukaryotic, prokaryotic and archaea cells [17][18][19]. In Eukaryotes, EVs are named based on their size, function, and biogenesis. Apoptotic bodies are very large vesicles with an average diameter of 1000–5000 nm, formed through the disassembly of the cell membrane. Ectosomes, also known as microvesicles, exhibit 100 to 1000 nm of diameter and are formed by the shedding of the plasma membrane. Exosomes are smaller vesicles ranging from 40 to 150 nm and originating from endosomal generation. Despite this categorization, the International Society for Extracellular Vesicles (ISEV) recommends the use of “extracellular vesicles” as a communal term for “particles naturally released from the cell that are delimited by a lipid bilayer and cannot replicate” [20][21][22]. These spherical structures play an essential role in cell communication by acting as biological carriers between different cells and tissues. Moreover, surface molecules on EVs can target specific cells and tissues, providing specificity to the recipient cell. The cargo includes acid nucleic molecules, such as DNA and RNA, proteins, lipids, enzymes, and any other molecules found in the donor cell.

EVs exert a physiological role in cellular communication but have also been found to be important drivers in the pathophysiology of many diseases, including AD in recent years. Thus, the mechanism of action by which microbe-derived EVs may contribute to immune modulation, the delivery of virulence factors, the enhancement of antibiotic resistance, as well as the facilitation of biofilm production, thereby impacting the physiopathology of inflammatory diseases [20], are still being uncovered.

2. Role of SA-Derived EVs in AD

In 2011, Hong et al. [23] conducted the first study to propose the role of *S. aureus*-derived EV in AD pathophysiology. The researchers reported that applying SA-derived EVs in tape-stripping mouse skin resulted in skin inflammation similar to that observed in AD patients, displaying a profile encompassed by (i) thickened epidermis, (ii) dermal infiltration of polymorphonuclear cells, (iii) an increase in Th1/Th17 inflammatory cytokines, and (iv) elevated serum IgE [23]. Importantly, these modifications were observed without the presence of live bacterial cells [23].

S. aureus colonization and infection evoke an immune response from the host by secreting pathogenic molecules and toxins (reviewed in Seiti Yamada Yoshikawa et al., 2019) [24]. Among these, α-hemolysin is emphasized, a cytotoxic protein secreted by *S. aureus* which induces cell death [25] on different cell types [26][27], including keratinocytes [28]. *S. aureus* from colonized AD skin releases greater amounts of α-hemolysin compared to *S. aureus* from the skin of healthy individuals. Furthermore, there are reports suggesting *S. aureus* α-hemolysin production is linked to AD severity [29].

An in vitro analysis revealed that EVs-associated α-hemolysin was more cytotoxic, and more effective to induce HaCaT keratinocytes death than soluble α-hemolysin, producing necrosis and inducing epidermal thickening and eosinophilic inflammation in the dermis [29][30], which could be explained by their better delivery to the host cell when encapsulated inside EVs. In addition, despite in vivo studies that indicate that soluble and EV-associated α-hemolysin induced skin barrier disruption and epidermal hyperplasia, an EV-associated toxin was the responsible for the induction of dermal infiltration [29].

Some *S. aureus* strains, such as ATCC14458 [23], a related cytotoxin producer, can release cytotoxins into the lumen of the EVs and induce their secretion to the extracellular space. Besides the assistance in the killing of the host cells by transferring these cytotoxic factors inside EVs, which favors their integration into the target cell cytoplasm [29], their association also shields the toxins from neutralization by the host immune system [23][29][31].

Mutually, in vitro and in vivo studies indicate that SA-derived EVs can upregulate pro-inflammatory mediators that elicit the Th17 response with augmented production of IgE, triggering AD-like inflammation [21][23]. Although AD is a disease ruled by type 2 immune responses (Th2, IL-4 and IL-13), IL-17 contributes to the worsening of the symptoms by enhancing IL-4 production from Th2 cells [32]. Moreover, SA-derived EVs induce the secretion of CXCL8 and TNF-α by primary human keratinocytes, the recruitment of neutrophils, and the formation of neutrophil extracellular traps, leading to better *S. aureus* skin colonization. The stimulation of CXCL8 is TLR2- and NFκB-dependent, and the induction level positively correlates with the membrane lipid and protein A in a similar quantity as those from pathogenic *S. aureus* [33].

SA-derived EVs can mediate inflammatory responses in AD pathogenesis; Kim et al. [34] examined the effect of these EVs on human dermal microvascular endothelial cells (HDMECs). HDMECs treated with SA-derived EVs increased the expression of cell adhesion molecules such as E-selectin, VCAM1, and ICAM1, and IL-6 with improved recruitment of monocytes in a TLR-4/NFκB-dependent signaling pathway [34]. All these findings together suggest that SA-derived EVs are key molecules in AD pathogenesis and are potential therapeutic targets. **Figure 1** illustrates the role of SA-derived EVs in AD and some key biological effects are summarized in **Table 1**.

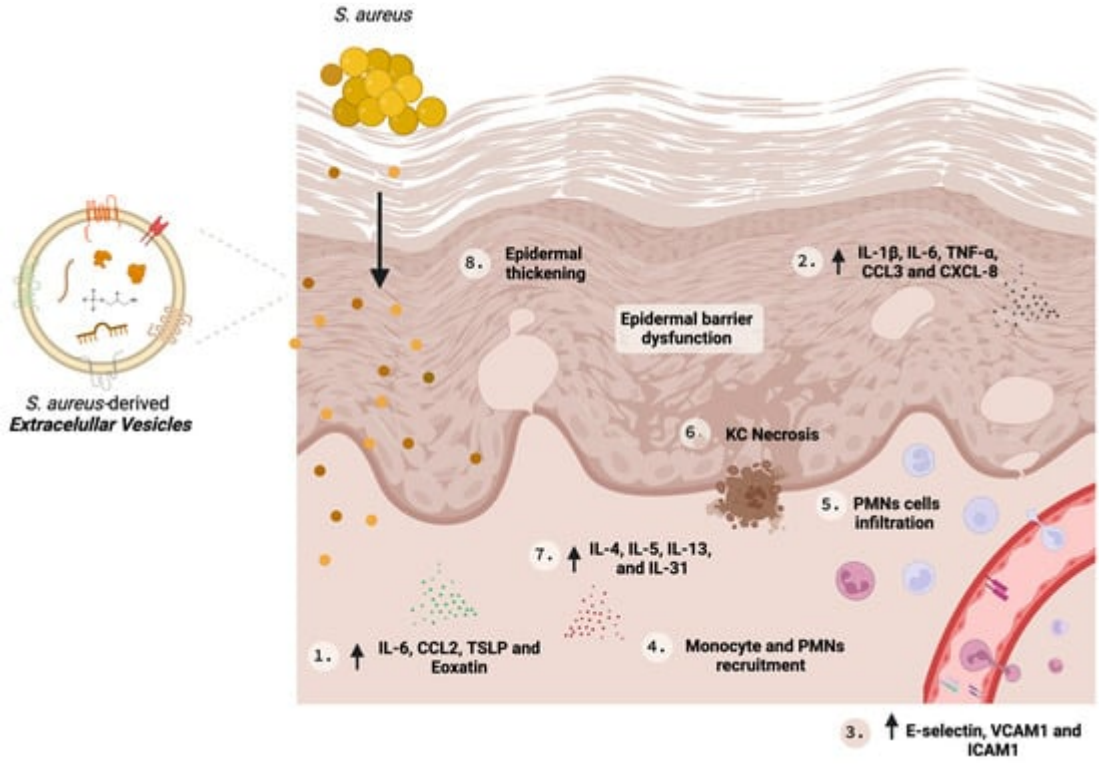

Figure 1. *S. Aureus*-derived EVs in AD pathogenesis. The effects of *S. aureus*-derived extracellular vesicles on skin cells are diverse. They can induce fibroblasts to secrete IL-6, CCL2, TSLP, and Eotaxin (1), and keratinocytes (KC) to secrete CXCL-8, IL-6, IL-1β, and TNF-α (2), all of which are proinflammatory mediators. Dermal microvascular endothelial cells upregulate the expression of the adhesion molecules E-selectin, VCAM1, and ICAM1 (3), which contribute to leukocyte migration (4). Secretion of CXCL-8 by KC also contributes to the cell migration, resulting in an increased polymorphonuclear cell (PMN) infiltration (5). Additionally, *S. aureus*-derived EVs can have a cytotoxic effect on KC, culminating in necrosis and skin barrier disruption (6). All these events together lead to eosinophilic inflammation in the dermis (7), epidermal hyperplasia (8), upregulation of proinflammatory mediators that elicit the Th2/Th17/Th1 response and augmented production of IgE. ↑: increased expression. Figure created with BioRender.com (accessed on 9 February 2024).

Table 1. Biological effects of SA-derived EVs.

S. aureus **Strain**	**Study Type**	**Experimental Model**	**Inflammatory Effector Molecules Upregulated**	**Histological Features**	**Others Observed Effects**	**Ref.**
ATCC14458	In vivo	EV were applied by tape stripping mouse skin	IL-4, IL-5, IL-17, IFN-γ	Infiltration of polymorphonuclear cells and epidermal thickness	-	[23]
03ST17	In vivo	Topical application of EVs into DFE induced lesions	IL-13, IL-31, CXCL8, CCL2 and CCL3	Infiltration of polymorphonuclear cells and epidermal thickness	Severe eczematous dermatitis, swelling,	[35]

***S. aureus* Strain**	**Study Type**	**Experimental Model**	**Inflammatory Effector Molecules Upregulated**	**Histological Features**	**Others Observed Effects**	**Ref.**
		on AD-like mouse model;			redness, bullae, and eschar formation	
USA300	In vitro	Primary human keratinocytes	CXCL8 and TNF-α	-	Recruitment of neutrophils and induction of NETs	[33]
ATCC 6538	In vitro	Immortalized human dermal microvascular endothelial cells	E-selectin, VCAM1, ICAM1 and IL-6	-	Recruitment of monocytes	[34]
ATCC14458 and from AD patients	In vitro	Immortalized human keratinocytes	IL-1β and IL-6	-	Cytotoxic effect associated with EV-α-Hemolysin	[29]
ATCC14458	In vitro	Primary mouse dermal fibroblasts	IL-6, TSLP, CCL2 and Eotaxin	-	-	[23]
03ST17	In vitro	Immortalized human keratinocytes	IL-6	-	-	[35]

S. aureus: *Staphylococcus aureus*; AD: atopic dermatitis; EV: extracellular vesicles; NETs: neutrophil extracellular traps; Ref.: References; DFE: *Dermatophagoides farinae* extract.

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