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# A new perspective in sepsis treatment: could RGD-dependent integrins be novel targets?

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**Sepsis is a life-threatening condition caused by the response of the body to an infection, and has recently been regarded as a global health priority because of the lack of effective treatments available. Vascular endothelial cells have a crucial role in sepsis and are believed to be a major target of pathogens during the early stages of infection. Accumulating evidence suggests that common sepsis pathogens, including bacteria, fungi, and viruses, all contain a critical integrin recognition motif, Arg-Gly-Asp (RGD), in their major cell wall-exposed proteins that might act as ligands to crosslink to vascular endothelial cells, triggering systemic dysregulation resulting in sepsis. In this review, we discuss the potential of anti-integrin therapy in the treatment of sepsis and septic shock.**

## Sepsis

Sepsis is a life-threatening clinical syndrome defined by a dysregulated host response to infection that can result in multiorgan failure [1]. In 2017, the World Health Assembly and WHO voted unanimously to declare sepsis a global health priority [2]. Although the true burden of sepsis remains unknown, current figures suggest that there are 48.9 million cases of sepsis annually, with over 11 million deaths worldwide [3]. Additionally, there is a strikingly higher burden among individuals living in areas with a lower socio-demographic index. Sepsis is now considered a leading cause of death worldwide, surpassing cancers and neoplasms (9.6 million), respiratory disease (3.9 million), Alzheimer's disease (1.7 million), and type 1 and 2 diabetes mellitus (1.4 million) [3,4].

Sepsis can originate from any site within the host, and only requires the presence of an invading microorganism with sufficient burden and virulence traits. A prevalence study across intensive care units in 75 countries reported that, in culture-positive patients, 62% were Gram-negative bacteria, 47% were Gram-positive bacteria, and 19% were fungal [5,6], with viruses having the lowest etiology. The most common Gram-negative isolates were

*Escherichia coli*, *Klebsiella* sp., and *Pseudomonas aeruginosa*; Gram-positive microorganisms included *Staphylococcus aureus* and *Streptococcus epidermidis*. The incidence of fungal infections in sepsis is rapidly rising and is predominantly caused by *Candida albicans*, with increasing numbers of cases involving *Candida auris* and *Aspergillus* sp. infections [5,7]. The most common sites of infection are the respiratory tract (63%), followed by abdominal (20%), bloodstream (15%), genitourinary (14%), skin (7%), catheter-related (5%), and central nervous system (3%) [5,6].

## Microvascular dysfunction in sepsis

Vascular endothelial cells are a highly adaptive and metabolically active single cell barrier that constantly sense alterations in the local extracellular environment [8]. Responsible for regulating homeostasis between the vessel wall and circulating blood, endothelial cells are frequently exposed to myriad signalling mediators. Cell communication and barrier maintenance rely on tight junction and adherens junction proteins, which are responsible for the permeability of solutes and ensuring vascular integrity, respectively [9]. Specifically, VE-cadherin localised across the basal membrane is crucial in supporting barrier permeability and has a major role in sepsis pathology [10].

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Microvascular dysfunction is well established during the early stages of sepsis and accounts for much of the pathology of sepsis and septic shock [11,12]. Upon binding to the vascular endothelium, pathogens, inflammatory cells and mediators lead to the loss of integrity of adherens junctions and increased paracellular permeability, with subsequent impairment of endothelial barrier function. Accumulation of oedema in tissues contributes to tissue hypoxia because of increased diffusion distances between functional capillaries and tissue cells in combination with poor oxygen solubility and transport in tissue water [8]. Therefore, microbes strategically disrupt the barrier integrity to trigger a dysregulated localised response, allowing for their escape out of the bloodstream. This promotes the development of secondary infections and multiple organ failure, including acute lung injury, renal, and cardiovascular dysfunction. Although efforts are being made to understand the dynamics of disease progression in sepsis, recent observations have suggested integrins expressed across the vasculature have a crucial role in the early stages of host–microbe interactions.

## Integrins

Integrins are noncovalently linked  $\alpha\beta$  heterodimers widely expressed on epithelial, endothelial, and immune cell surfaces [13,14]. These adhesion molecules mediate cell crosstalk between these cells and secure them to the extracellular matrix (ECM) [14]. However, aside from their crucial role in maintaining structural integrity, they are also coupled to members of the Focal Adhesion Kinase (FAK) family [12–14]. Integrin engagement across the FAK proteins controls tyrosine activation and phosphorylation, which regulate downstream events that allow for functional regulation of host activities, including cytoskeleton rearrangement, immune mediator secretion, immune cell recruitment, extravasation, or regulating structural integrity [13]. Therefore, integrins are crucial to modulating host defence, and have a major role in sepsis pathology.

## RGD recognition sites in integrins

Integrins also convey a plethora of bidirectional signal transduction events, referred to as either ‘inside-out’ or ‘outside-in’ signalling. The former results in activation or deactivation of integrins initiated by an internal cellular pathway. In the latter, conforma-

tional changes are induced upon ligand binding that elicit intracellular signalling, leading to a downstream response that can alter biological processes [12,14]. Integrins are classified based on their ligand-recognising motifs. The most common subset bind to the RGD sequences [15,16]. Most ligands retaining this motif are located in the ECM, and act as adhesion molecules to anchor endothelial cells to the ECM, providing structural integrity. These components include collagen, fibrinogen, fibronectin, vitronectin, and laminin [15]. Some proteins, such as fibrinogen and vitronectin, are of particular importance because of their dual presence in the plasma; they are associated with regulating homeostasis in the coagulation cascade. The polarised endothelial barrier is highly populated with RGD-recognising integrins on its abluminal and luminal surfaces. These include  $\alpha5\beta1$ ,  $\alphaV\beta1$ ,  $\alphaV\beta3$ ,  $\alphaV\beta5$ ,  $\alphaV\beta6$ ,  $\alphaV\beta8$ , and  $\alpha8\beta1$  (Table 1). The  $\beta1$  integrins represents the largest subgroup. Specifically,  $\alpha5\beta1$  is an RGD-recognising integrin, and has high specificity for fibronectin [17]. Its expression in endothelial cells is regulated by the growth factor fibroblast growth factor (FGF) and, therefore, the inflammatory mediator tumour necrosis factor (TNF)- $\alpha$  is implicated in infection [18]. Some integrins vary in their ability to recognise ligands, where the most promiscuous subtypes include the  $\beta3$  integrins, capable of interacting with a large number of ECM and plasma proteins. Specifically, endothelial  $\alphaV\beta3$  recognises vitronectin, fibrinogen, fibronectin, and von Willebrand factor (vWF), each of which contains the classic RGD motif [19,20]. Its expression on endothelial cells is upregulated in the presence of TNF $\alpha$  secreted during infection [21]. Clearly, the lack of specificity and host-wide expression makes this integrin a desirable target for pathogens. By contrast, some RGD-recognising integrins are more stringent in their ligand preference; endothelial  $\alphaV\beta5$  and  $\alphaV\beta8$  have high specificity for vitronectin [15,22].

This tripeptide is the minimal requirement to engage an RGD-binding integrin [16]. Recent observations reported a growing number of microorganisms (bacterial, fungal, and viral) recognising or harbouring RGD-motifs, which in turn can crosslink to host integrins. Given that these multifunctional receptors also regulate intracellular signalling through the FAK system, microbes can exploit them to encourage various stages of pathogenic infection. Therefore, this clearly implicates RGD-binding integrins in the pathophysiology of human disease. In this review, we explore how

TABLE 1

### Distribution of RGD-binding integrins and their major ligands<sup>a</sup>

RGD-binding integrin	RGD-containing ligands	Major cell/tissue distribution	Refs
$\alphaV\beta1$	Fibronectin, vitronectin, laminin	Fibroblasts	[97]
$\alphaV\beta3$	Fibrinogen, fibronectin, vitronectin, vWF	Lung and vascular endothelium, alveolar and large airway epithelium, platelets, fibroblasts	[19–21]
$\alphaV\beta5$	Vitronectin, fibrinogen, fibronectin	Lung and vascular endothelium, epithelium, fibroblasts	[16]
$\alphaV\beta6$	Vitronectin, fibrinogen, fibronectin	Epithelium	[98]
$\alphaV\beta8$	Vitronectin	Fibroblasts, lung epithelium	[22]
$\alpha5\beta1$	Fibronectin	Lung and vascular endothelium	[17]
$\alpha11\beta3$	Fibrinogen, fibronectin, vWF	Platelets	[99]
$\alpha8\beta1$	Fibronectin	Embryonic neurites, fibroblasts	[100]

<sup>a</sup> Integrins that recognise the RGD motif are the most common subtype and are widely expressed across the surface of epithelium, endothelium, and immune cells. Ligands that contain the RGD sequence are found in the interstitial space that surrounds the abluminal side of the endothelium, or the serum that flows within blood vessels.

RGD-dependent integrins have emerged as attachment and internalisation receptors for various classes of pathogen-mediated sepsis, specifically induced by bacteria, fungi, and viruses.

### Bacterial-induced sepsis

Bacterial pathogens of humans have evolved a range of virulence factors to promote motility, attach to epithelial or endothelial cell surfaces, avoid host immune responses, activate or inactivate host cellular pathways, and ultimately cause disease. Toxins secreted by Gram-negative and positive bacteria trigger an immune response, which results in dysregulation of the carefully synchronised equilibrium between pro- and anti-inflammation. Lipopolysaccharide (LPS) is a conserved component of the outer membrane in Gram-negative bacteria and is arguably the most potent immunostimulants implicated in the bacterial pathogenesis of septic shock. It induces profound reactions by activating lethal coagulation and complement cascades, as well as triggering the release of the proinflammatory cytokines interleukin (IL)-6, IL-8, and IL-1 $\alpha/\beta$  upon immune cell recognition [23].

Pathogens heighten their interplay with host cells by harbouring numerous adhesion mechanisms. This allows for prolonged contact, which significantly stabilises their attachment and encourages subsequent invasion strategies. *S. aureus* expresses a plethora of such surface proteins, including Microbial Surface Components Recognising Adhesive Matrix Molecules (MSCRAMMs) [24]. One of which, Clumping Factor A (ClfA), recognises plasma and extracellular components vWF and fibrinogen [25]. These host plasma proteins contain the conserved RGD motif, which is essential for recognition by RGD-binding integrins (Fig. 1). *S. aureus* crosslinks these proteins to indirectly bind integrins, which facilitates adherence and internalisation. Although the explanation behind this distant bacterial attachment remains elusive, it might allow for host–pathogen interactions at a safe distance, avoiding an immune response that could trigger bacterial clearance.

A shear-based infection model demonstrated that *S. aureus* ClfA binds fibrinogen, which crosslinks to the major RGD-dependent integrin expressed on endothelial cells,  $\alpha$ V $\beta$ 3 (Fig. 2a) [26]. This interaction results in cell activation, leading to vWF deposition on the endothelial surface, which further enhances bacterial attach-

ment. In turn, numerous downstream signals result in endothelial cell apoptosis and reduced expression of VE-cadherin. This leads to a significant reduction in vessel wall integrity and increased vascular permeability, which facilitates bacterial escape from the blood vessel into nearby tissue [30,31,40–42]. Once adhered, these bacteria are exposed to the fluid dynamic and shear environment, which induces platelet recruitment. Kerrigan *et al.* demonstrated that adhered *S. aureus* ClfA binds plasma fibrinogen and IgG, which crosslink to the major platelet integrins  $\alpha$ IIb $\beta$ 3 and Fc $\gamma$ RIIa, respectively [27–30]. This engagement triggers platelet activation, granule secretion, amplification of the response, and thrombus formation on the endothelial surface. These are crucial aspects in the evasion strategy of *S. aureus*, because bacteria encased in a growing thrombus are safe from circulating immune cells or antibiotics.

To further strengthen its position, *S. aureus* internalises itself into vascular endothelial cells by exploiting another host integrin,  $\alpha$ 5 $\beta$ 1 [31]. This occurs when *S. aureus* coats itself in fibronectin or fibrinogen through the Fibronectin Binding Protein A/B (FnBPA/B), a key MSCRAMM, forming a crosslink to the endothelial cell integrin  $\alpha$ 5 $\beta$ 1 [23,32,33]. This is a highly controlled event triggering a cytoskeletal protein signalling cascade, involving F-actin rearrangement, tyrosine phosphorylation, mitogen-activated protein kinase activation and Src family kinase activation [30,32–34]. Although the motive behind this action remains obscure, immune cells and antibiotics are unable to penetrate endothelial cells, which suggests that *S. aureus* internalises in the host to evade these attacks. This is of significant concern because *S. aureus* can re-emerge after inflammation subsides, causing re-infection or recurrence of sepsis, which is common in patients [35].

Several other Gram-positive bacteria that induce sepsis are also affiliated with integrins. The major cell wall protein PspC of *Streptococcus pneumoniae* binds the plasma protein vitronectin and crosslinks the bacterium to the integrin  $\alpha$ V $\beta$ 3 [36]. This interaction also facilitates attachment and internalisation into lung epithelial cells in a similar manner to *S. aureus* [36]. Although less commonly isolated, *Streptococcus pyogenes* can also cause sepsis. It expresses a fibronectin-binding protein SfbI, which binds fibronectin and crosslinks the bacterium to epithelial cells via integrin  $\alpha$ 5 $\beta$ 1, which facilitates internalisation [37,38].

Fibrinogen ( $\alpha$ chain)	-----LTTNIMEIL <b>RGDF</b> --SSANNRDNT----YNRVSE--DLR-SR
Fibronectin	GIILAALGGDVE--- <b>KRGDR</b> ---EEAHVPFFSVMLIGGN-----
Vitronectin	--LEGCFPPGLYMDER <b>RGDC</b> --VPKAQCPCY----YDGE-----IF
von Willebrand factor	CTDYTAECKPQV--- <b>TRGDVFTMP</b> EDEYTVY----DDGE-----E
Laminin ( $\alpha$ 1 chain)	GVDYTITVYAVT--- <b>GRGDS</b> ---PASSKPIS----INYTE-----ID
Collagen ( $\alpha$ 1 chain)	--GAAGLPGPKG--- <b>DRGDA</b> --GPKGA-----DGSPGKDGVRGLT
	***

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FIGURE 1

Sequence alignment of common extracellular matrix and plasma proteins that share the Arg-Gly-Asp (RGD) motif. Protein sequences and their UniProt unique protein identifier (UPI) in respective top–bottom order (P02671, P02751, P04004, P04275, P25391, and P02452). The RGD sequence is highlighted in bold. The MAFFT program was used for multiple sequence alignment.

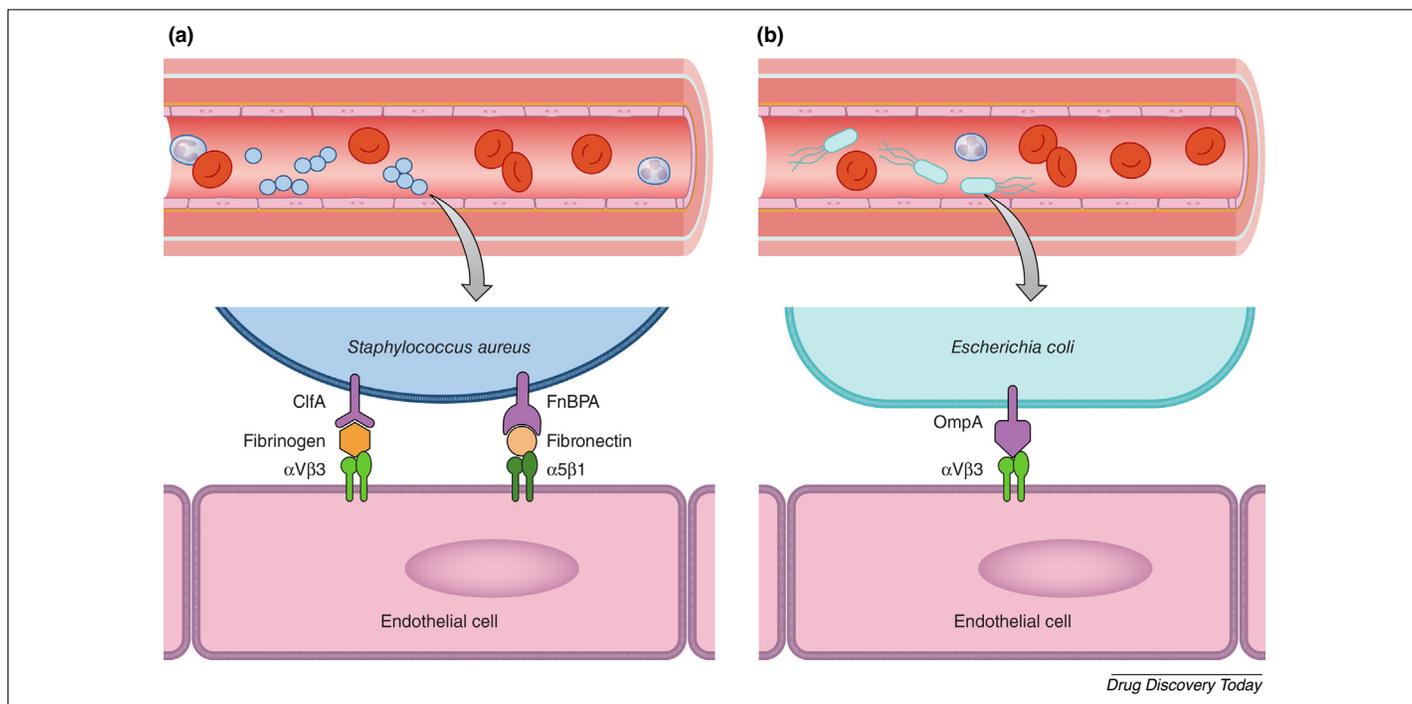


FIGURE 2

Bacterial interaction with vascular endothelial cells. **(a)** The Gram-positive bacterium, *Staphylococcus aureus*, indirectly interacts with the endothelium through extracellular matrix components to promote colonisation and pathogenesis. Clumping Factor A (ClfA) recognises fibrinogen, the Arg-Gly-Asp (RGD)-containing ligand of host  $\alpha V\beta 3$ . This mediates adherence. Additionally, fibronectin-binding protein A (FnBPA) recognises fibronectin, an RGD-containing ligand that is recognised by host integrin  $\alpha 5\beta 1$ . This mediates internalisation into the endothelial cell. **(b)** The Gram-negative bacterium, *Escherichia coli*, comes into direct contact with the endothelium without requiring an extracellular matrix crosslink. Outer membrane protein A (OmpA) contains an RGD motif to interact with the RGD-recognising integrin,  $\alpha V\beta 3$ . This promotes adherence to the endothelial cell.

Several Gram-negative bacteria, including *E. coli* and *Pseudomonas aeruginosa*, are also commonly isolated from patients with sepsis and engage with integrins to promote infection [26,39]. Recently, McHale *et al.* demonstrated that, in contrast to *S. aureus*, ECM proteins are not mandatory for *E. coli* to interact with endothelial cells (Fig. 2b) [40]. In a shear-based infection model, they revealed that this bacterium expresses an RGD-containing surface molecule called Outer Membrane Protein A (OmpA), which binds directly to endothelial cell integrin  $\alpha V\beta 3$ . A strain deficient in OmpA or blocking  $\alpha V\beta 3$  significantly reduced this interaction [40]. Binding resulted in endothelial dysfunction and vascular leakage through induction of endothelial cell apoptosis and loss of the adherens junction protein, VE-cadherin [40]. Similarly, *P. aeruginosa* expresses an RGD motif in the PilY1 pilus-associated protein, which binds epithelial  $\alpha V\beta 5$  and  $\alpha V\beta 3$  through the plasma protein vitronectin [41]. Thus, integrin receptors are shared targets of various bacterial families, which raises the intriguing notion of targeting the host rather than the pathogen for sepsis treatment.

### Fungal-induced sepsis

Invasive fungal infections contribute substantially to human morbidity and mortality, particularly in immunocompromised patients [5]. *Candida* spp. remain the predominant cause of invasive candidiasis, and *C. albicans* accounts for 66% of all cases [42].

*C. albicans* exists as a polymorphic fungus, capable of reversibly transitioning between yeast, pseudohyphal, and hyphal forms; this trait considerably promotes its pathogenicity in the host. Both yeast and hyphae are crucial for stages of disease, because mutants

unable to switch are avirulent, suggesting that morphological plasticity substantiates its potency [43–45]. However, before either form is introduced into the bloodstream, they must first adhere to the vasculature, where they are enveloped by ECM proteins, including fibrinogen, fibronectin, and vitronectin. After successful entry across the endothelium, *C. albicans* uses diverse evasion strategies that both encourage host dysregulation and promote its own persistence and dissemination. Both yeast and filamentous growth can be found in the bloodstream during systemic candidiasis, and their collaborative efforts are crucial in maintaining pathogenicity [46]. Upon contact with immune cells, yeast cells are recognised and phagocytosed by macrophages, but then switch into hyphae to pierce and kill the immune cell, allowing their escape [47]. Both morphologies are capable of inducing various immune responses of host cells: hyphae interact with host adhesins that coat the surface of endothelial cells to induce endocytosis and, in turn, release TNF- $\alpha$ , IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, and IL-8 [48–50]. Yeast cells induce a stronger cytokine response from peripheral blood mononuclear cells, likely because of a high density of cell wall pathogen-associated molecular patterns (PAMPs): O-mannan, N-mannan, and  $\beta$ -glucans [51–53]. A crucial *in vitro* circulatory system examined the *Candida*-endothelium interaction under flow and revealed that both yeast and hyphae were capable of adhering to the vasculature [54–56]. Longer germ tubes experienced higher shear stress and failed to maintain prolonged contact with the host, whereas yeast cells with smaller volume or hyphae with short germ tubes could successfully anchor [54–56]. Adhesins reportedly involved with hyphae endocytosis into the endotheli-

um are fungal Als3 and host cadherins; however, it is also speculated that *C. albicans* has evolved an Als-independent mechanism to adhere and invade host cells [50].

Although no studies have investigated whether *C. albicans* directly induces vascular leakage, transcriptome profiles of infected endothelial cells reveal upregulation of pathways involved in angiogenesis, apoptosis, and inflammatory response [48]. Furthermore, fungal cells are capable of decreasing epithelial barrier integrity to promote their translocation through a transcellular route [57].

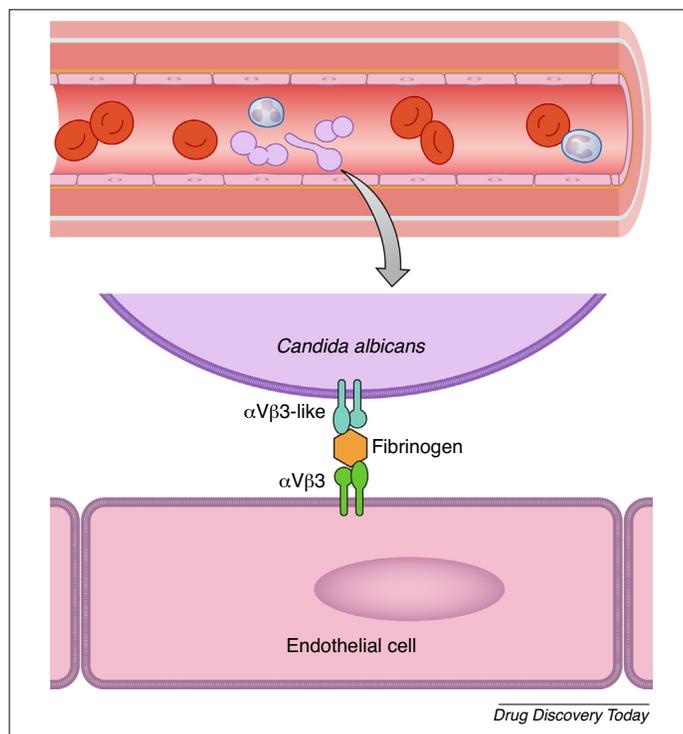
Despite *C. albicans* displaying high contact with the vasculature, a paucity of information still surrounds its mechanism of adherence and its capability to induce endothelial dysfunction. In a shear-based infection model under conditions of flow (0.25 dynes  $\text{cm}^{-2}$ ), yeast and hyphal cells bound significantly to endothelial cells [54–56]. This low flow rate is observed among the microvasculature, signifying a prime location for fungal infiltration. These postcapillary venules are most susceptible to infection because they express both integrins and intracellular junction proteins that can be manipulated by the fungus to encourage a paracellular route of infection [58].

Interestingly, *C. albicans* expresses several surface proteins that are conserved from humans [59]. These homologous genes might have evolved to enhance colonisation, evade host immunity, or act as virulent factors to promote dissemination. For example, studies suggested that *C. albicans* expresses integrin-like receptors on its surface [56,60–63], most notably  $\alpha\text{V}\beta 3$ ,  $\alpha\text{V}\beta 5$  and  $\alpha\text{V}\beta 1$ . These integrin-like surface proteins are capable of binding human plasma proteins, such as fibrinogen, fibronectin, and vitronectin, and adherence to the host could be inhibited by human monoclonal and polyclonal antibodies against  $\alpha\text{V}\beta 3$ ,  $\alpha\text{V}\beta 5$ , and  $\alpha\text{V}\beta 1$  [62,64–67]. These results suggest that an integrin analogue present on the fungal surface is capable of mediating adhesion to the host. However, it remains elusive whether *C. albicans* uses this unique strategy of plasma protein–integrin interaction to crosslink with human host cells to initiate infection (Fig. 3).

### Viral-induced sepsis

Viruses represent the third leading cause of sepsis and account for nearly 4% of co-infections [5]. Viruses typically attack the respiratory mucosa, where they induce an intense inflammatory response to trigger acute lung epithelial and endothelial injury. This promotes secondary infections, which likely drive sepsis and prolong severe host dysregulation.

Similar to mechanisms used by other sepsis pathogens, several viruses express RGD motifs, which bind integrins, to allow for attachment or entry to host cells (Fig. 4). Human metapneumovirus (HMPV) causes a spectrum of respiratory illnesses ranging from mild colds to severe pneumonia. Its surface fusion protein carries an RGD motif, responsible for virus–host adhesion and entry [68]. This interacts with integrin  $\alpha\text{V}\beta 1$  to confer binding of HMPV, even in the absence of its primary attachment glycoprotein [68]. Adenovirus serotypes 2 (Ad2) and 5 (Ad5) are respiratory viruses that contain RGD sequences in their penton base proteins, which promote a multistep entry process [69]. Viral RGD expression interferes with the ability of integrin to connect host cytoskeleton to ligands of the ECM. Therefore, high-affinity interactions between penton proteins and  $\alpha\text{V}\beta 3$ ,  $\alpha\text{V}\beta 5$ , and



**FIGURE 3**

Fungal interactions with vascular endothelial cells. Fungi, such as *Candida albicans*, indirectly interacts with the endothelium through extracellular matrix components to promote colonisation and pathogenesis. *C. albicans* putatively expresses an  $\alpha\text{V}\beta 3$ -like surface protein, analogous to that of human endothelial cells. It recognises fibrinogen, which crosslinks with host  $\alpha\text{V}\beta 3$ , thus mediating its adherence to the endothelial cell.

$\alpha\text{V}\beta 1$  promote cell detachment from the ECM (Fig. 5). Blocking these integrins prevents viral entry, suggesting that this pathogen is considerably dependent on its RGD motifs [69–71]. A member of the subfamily *Betaherpesvirinae*, Human cytomegalovirus (HCMV) causes severe complications in immunocompromised and fetal newborns. It induces  $\alpha\text{V}\beta 6$  expression in endothelial cells, which activates transforming growth factor (TGF)- $\beta 1$  and triggers a downstream signalling cascade resulting in cell proliferation, migration, and ECM synthesis [72]. Several abundant HCMV proteins, including M44, UL148, UL30, and US23, express RGD sequences; these might interact with integrins to serve as receptors for viral endocytosis. Epstein–Barr virus (HHV-4 or EBV) primarily targets epithelial cells, and its BMRF2 envelope protein contains an RGD motif that facilitates adhesion to  $\beta 1$  integrins [73]. Two pathogens in the *Picornaviridae*, Human Parechovirus 1 (HPeV1) and Coxsackievirus A9 (CV-A9), encode RGD sequences in capsid proteins that bind  $\alpha\text{V}\beta 3$  and  $\alpha\text{V}\beta 6$  to allow for viral entry [74–75].

During late 2019, outbreaks of lethal pneumonia were reported across China [76]. By March 2020, the causative viral agent, severe acute respiratory syndrome-related coronavirus (SARS-CoV-2), was declared a global pandemic [77]. The coronavirus family is a family of highly pathogenic zoonotic viruses. Its phylogenetic tree revealed that the novel SARS-CoV-2 virus shares higher sequence similarity to SARS-CoV, than to Middle Eastern respiratory syndrome coronavirus (MERS-CoV), to which it is more distantly related [78]. They share major structural proteins; one of which, the spike (S) surface glycoprotein, is of particular interest because

SARS-CoV-2	YA-----DSFV <b>IRGD</b> -----EVRQIAPGQGTGKIADYNYKLPD-DFT-----GC
Human megapneumovirus (hMPV)	PN-----EKDCET <b>RGD</b> -----HVFCDTAAGINVAEQ-SKECNI-NIS-----TT
Human adenovirus subtype 5 (Ad5)	QPVED---MNDHAI <b>IRGD</b> -----TFATRAEEKRAE---AEAAAE-AAA-----PA
Human cytomegalovirus (HCMV)	LQHVE---TLRRFL <b>RGD</b> -----SCFVHDLRGMMD---YHDGLS-RRQ-----QR
Epstein Barr Virus (EBV)	APKV---TSSRAL <b>RGD</b> -----MANLTNQSPYGGQ---PQNRMM-KLA-----YL
Human parechovirus 1 (HPeV1)	TVTS---IQSER- <b>RGD</b> -----MSALNTHGAFGGQ---SGAIYVGNYS-----VV
Coxsackievirus A9 (CV-9)	VILLVCFCAGAAHS <b>RGD</b> -----TFQTSSSPT-----PPGSSS-KAP-----TK
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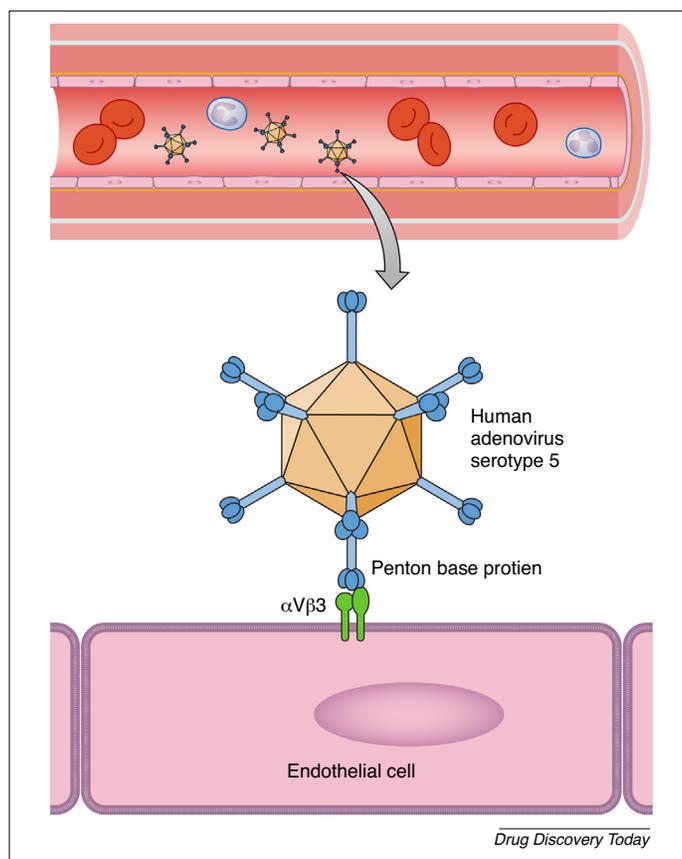
**FIGURE 4** Sequence alignment of viral proteins sharing the Arg-Gly-Asp (RGD) motif. The sequences of eight clinically important viruses were aligned based on the expression of surface proteins involved in the interaction with integrins, through expression of an RGD motif. The RGD region is highlighted in bold. The MAFFT program was used for multiple sequence alignment.

of its crucial role in permitting adhesion, fusion, and entry into the host. Current evidence suggests that adhesion of SARS-CoV-2 is first mediated through the receptor-binding domain (RBD) located in the S1 subunit of spike protein [79]. Furthermore, recent sequence alignment between SARS-CoV and SARS-CoV-2 illustrated that a distinctive K403R mutation in the spike protein creates an RGD motif within SARS-CoV-2 [80]. This is a crucial finding because it might implicate integrins in the attachment process given that Arg-Gly-Asp is the minimal peptide sequence required to bind RGD integrins. Various studies demonstrated that the integrins  $\alpha V\beta 3$ ,  $\alpha 5\beta 1$ , and  $\alpha V\beta 5$  are widely expressed across tracheal, bronchial, and alveolar epithelium as well as lung microvascular endothelium [38,81]. Of note,  $\alpha V\beta 3$  expressed on interalveolar capillaries compromises >95% of the lung vascular surface area [38]. Therefore, by expressing this motif, SARS-CoV-2 has evolved the ability to bind to integrins. Although the functionality behind this mutation remains elusive, it might have evolved to bring the virus into close proximity with its true entry target, angiotensin-converting enzyme (ACE)-2, and secure attachment to host.

**Integrins as targets for sepsis**

The functionality of integrins are dependent on several processes, including ligand engagement, downstream signal transduction, and cytoskeletal response. Inhibition of any of these events inhibits fundamental cell biological processes and, therefore, present as an attractive pharmacological target. The interaction between integrins with their ligands are being explored for their use as therapeutics, and investigations that target the crucial Arg-Gly-Asp motif have proven to be the most successful [82–84]. Antibodies, linear and cyclic peptides, peptidomimetics, disintegrins, and small-molecule antagonists have all been designed to bind to the target integrin in a similar manner to its natural ligand, which prevents ligation or displaces bound ligands.

Therapeutic antibodies are the most successful molecules in integrin-based therapy. Currently, they are under investigation in preclinical and clinical trials to target a multitude of illnesses, including Crohn’s disease, psoriasis, rheumatoid arthritis, and acute coronary syndromes [85–88]. A major advantage of developing therapeutic antibodies is that they exhibit high target specificity, with few off-target sites. Binding specificity and affinity can be regularly modified, and their long half-life make



**FIGURE 5** Viral interactions with vascular endothelial cells. Representing viruses, human adenovirus serotype 5 directly comes into contact with the endothelium without requiring an extracellular matrix crosslink. Its penton base proteins express an Arg-Gly-Asp (RGD) motif that is recognised by the RGD-recognising integrin,  $\alpha V\beta 3$ . This promotes adhesion and entry of the virus.

these molecules highly desired [89]. However, when being developed for the treatment of infection, full-length antibodies cannot be used because many bacteria express antibody-binding proteins (i.e., *S. aureus* Protein A, SpA, or Second Immunoglobulin-Binding protein, SBI) that bind to Fc portions of antibodies and, therefore, could exacerbate the infection response in the host [90,91].

TABLE 2

**Surface proteins expressed by human pathogens that promote various stages of infection by interacting with host RGD-recognising integrins**

Microbial agent	Host integrins	Host response	Refs
<b>Bacteria</b>			
<i>Staphylococcus aureus</i>	$\alpha V\beta 3$ , $\alpha 5\beta 1$	Adhesion, vascular dysfunction, internalisation	[26,29,30,32–34,96]
<i>Escherichia coli</i>	$\alpha V\beta 3$	Adhesion, vascular dysfunction	[40]
<i>Pseudomonas aeruginosa</i>	$\alpha V\beta 3$ , $\alpha V\beta 5$	Adhesion	[41]
<i>Streptococcus pneumoniae</i>	$\alpha V\beta 3$	Adhesion, internalisation	[36]
<i>Streptococcus pyogenes</i>	$\alpha 5\beta 1$	Internalisation	[37]
<b>Fungi</b>			
<i>Candida albicans</i>	$\alpha V\beta 3^a$ , $\alpha V\beta 5^a$	Adhesion, vascular dysfunction <sup>a</sup>	[62–67]
<b>Virus</b>			
SARS-CoV-2	$\alpha V\beta 3^a$	Adhesion <sup>a</sup>	[80]
Human metapneumovirus	$\alpha V\beta 1$	Adhesion, entry	[68]
Human adenovirus subtype 5	$\alpha V\beta 1$ , $\alpha V\beta 3$ , $\alpha V\beta 5$	Adhesion, entry	[69–71]
Human cytomegalovirus	$\alpha V\beta 6$	Adhesion	[72]
Epstein–Barr virus	$\beta 1$ subtype	Adhesion	[73]
Human parechovirus 1	$\alpha V\beta 3$ , $\alpha V\beta 6$	Entry	[74]
Coxsackievirus A9	$\alpha V\beta 3$ , $\alpha V\beta 6$	Entry	[75]

<sup>a</sup> Speculated interactions and implications.

Peptide-based drugs are increasingly being investigated as therapeutic options for targeting integrins. These drugs contain binding motifs similar to sequences found in the endogenous ligand. Therefore, they are effectively recognised by integrins, and act as competitive antagonists against the natural ligand. Given that the crucial binding site for ligand interaction is well established, these drugs are relatively straightforward for drug design. However, some issues remain surrounding peptide-based drugs. Although they display moderate to high affinity, they can lack specificity because of shared interactive sites found in other integrins [92]. This can be addressed by adjusting the surrounding flanking sequences. Also, a significant concern of using peptide-based drugs is that they are sensitive to proteolytic cleavage *in vivo*. This instability can be resolved through cyclisation of the peptides or addition of D-amino acids that can protect against proteolytic degradation, hence prolonging the circulation half-life [89].

Finally, small-molecule antagonists are also effective drugs to target integrins. These molecules are low-molecular-weight synthetics that are easily manufactured and less costly than antibody or peptide-based drugs [92]. They tend to be zwitterionic in nature and present challenges with bioavailability, serum protein binding, and integrin selectivity [89,93].

The current challenge that clinicians face in sepsis is the joint issue of failed early diagnosis and ineffective treatment methods. Previous approaches have focussed on treating or controlling late-stage pathophysiological effects (such as inflammation, thrombus formation, coagulation, etc), an approach that has resulted in the failure of many compounds in clinical trials as a result of later intervention in the disease progression pathway. RGD-dependent integrins are evidently central to the pathology of sepsis. They are clearly a shared target of several pathogens, including bacteria, fungi and viruses, and are widely expressed across tissues, which merits integrin-focused therapy. This could represent a significant breakthrough in our understanding of the early interactions between pathogen and host. Specifically, integrins involved in the primary step of pathogen–host interaction, endothelial permeability, and vascular leakage are crucial in the prevention of sepsis. In particular,  $\alpha V\beta 3$  has been identified as a commonly targeted

receptor between major pathogenic aetiologies of sepsis to mediate adhesion or entry. These include *S. aureus*, *E. coli*, *P. aeruginosa*, human adenovirus, human parechovirus 1, Coxsackievirus A9, with putative interactions involving *C. albicans* and SARS-CoV-2 (Table 2). When targeted by these pathogens,  $\alpha V\beta 3$  serves as a modulator for mitigating vascular leak and endothelial permeability and anti- $\alpha V\beta 3$  therapeutics could have major implications in sepsis management. Additionally,  $\alpha V\beta 3$  integrins are widely expressed across the endothelium, which acts as the final barrier for microbe penetration. Hence, the development of  $\alpha V\beta 3$  antagonists appears lucrative. This is of particular interest in the current climate, where outbreaks and pandemics, such as SARS-CoV-2, spread globally, causing a surge in antimicrobial use and further paving the way for the development of superbugs. Given that sepsis is diagnosed in 100% of nonsurvivors infected with SARS-CoV-2, it is essential to radically accelerate the development of therapeutic agents [94]. The most advanced and widely investigated  $\alpha V\beta 3$ -inhibitor, cilengitide, is a cyclic peptide well documented for its treatment of glioblastoma [95]. Recent data demonstrated that *S. aureus* and *E. coli* interaction with the human endothelium was significantly reduced upon administration of cilengitide, under physiological conditions of shear stress and flow [40,96]. Therefore, it might be fruitful to investigate the potential of cilengitide in reducing pathogen–host interactions during the very early stages of sepsis progression. In light of this, renewed interest towards integrin antagonists is clearly developing, where combination regimens might be efficacious towards the clinical management of sepsis.

### Concluding remarks

Sepsis, a dysregulated host response to infection, remains one of the largest killers in the world, surpassing that of cancers, diabetes, and Alzheimer's disease. Late-stage sepsis is characterised by enhanced vascular permeability and leakage, subsequently promoting microbial escape into the bloodstream. This results in tissue oedema, hypoxia, and multiple organ failure. Inhibiting the dysregulation of the vascular endothelium by preventing initial pathogen attachment could reduce the risk of developing septic shock. In this review, we demonstrated a potentially new perspective in therapeutics against sepsis: integrin-

based therapy. With regards to regulating cell crosstalk, intracellular signalling, inflammatory responses, and structural integrity, the RGD-dependent integrin superfamily evidently has important roles in sepsis pathology. Evidence has since accumulated demonstrating that bacteria, viruses, and fungi exploit these receptors to crosslink host cells, in turn promoting adherence, entry, and further dissemination, resulting in septic shock. Therefore, these highly varied classes of pathogens share a common target, which presents a lucrative opportunity to use integrins as a therapeutic candidate against sepsis. Specifically, the  $\alpha V\beta 3$  receptor, widely expressed across the epithelium and endothelium, recognises a vast array of RGD-containing proteins present in the ECM. The host-wide expression and lack of specificity make it a

desirable target for several microbes within the bacteria, fungi, and viruses. Developing an integrin antagonist that competes against ECM for the RGD recognition site would thereby effectively prevent initial adherence of the pathogen to host. In turn, this would maintain endothelial barrier stability and hinder microbial dissemination, reducing the risk of mortality and promoting patient survival.

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