Role of β2 Integrins in Neutrophils and Sepsis

Koichi Yuki,a,b Lifei Hou,a,b

aDepartment of Anesthesiology, Critical Care and Pain Medicine, Cardiac Anesthesia Division, Boston Children’s Hospital, Boston, Massachusetts, USA
bDepartment of Anaesthesia, Harvard Medical School, Boston, Massachusetts, USA

ABSTRACT

Sepsis remains medically challenging, with high morbidity and mortality. A novel intervention is urgently needed in the absence of specific, targeted therapy. Neutrophils act as double-edged swords in sepsis; they can help to eradicate microbes, but they also contribute to tissue injury. β2 Integrins are critical adhesion molecules that regulate a number of neutrophil functions. β2 Integrins consist of four members, namely, αLβ2, αMβ2, αXβ2, and αDβ2. Here, we review the role of each β2 integrin in neutrophils and sepsis and consider future direction for therapeutic intervention.

KEYWORDS

sepsis, β2 Integrins, neutrophils

BACKGROUND

Although the medical field has continued to progress, sepsis remains a medically challenging disease. It is the leading cause of death in intensive care units (ICUs) and is the most expensive condition treated in the United States (1). Furthermore, its incidence has been increasing (2). Sepsis was originally defined as infection in the presence of systemic inflammatory response syndrome (SIRS). Under this definition, previous preclinical and clinical sepsis research had been centered on the identification and modulation of inflammatory targets. A number of anticytokine therapies were developed and tested, but none of them turned out to be clinically successful (3). Furthermore, a number of patients suffering from infection and significant organ failure did not meet the criteria of SIRS and were not diagnosed as having “sepsis,” despite high morbidity and mortality. Based on these facts, the original sepsis definition was challenged. In the third international consensus definition for sepsis and septic shock (Sepsis-3), sepsis was redefined as infection in the presence of life-threatening organ injury (4), where organ injury would be assessed by the sequential organ failure assessment (SOFA) score (5). The severity of organ dysfunction and the increased number of dysfunctional organs were associated with increased mortality (6, 7). The Sepsis Survival Campaign is a worldwide effort to standardize care of septic patients for better outcome, and it has been advocating early infection source control. Certainly, early antimicrobial therapies, irrigation and drainage (I&D), and surgical debridement are critical, but there is currently no direct therapy against organ injury. A clinical bundle by the Sepsis Survival Campaign has not yet shown a significant contribution to the improvement of outcomes (8, 9). With the conceptual shift of the condition of “sepsis,” therapeutic intervention other than anti-inflammatory therapies should be considered. Thus, it is important to understand factors that are responsible for organ injury (10). One of the difficulties of dealing with sepsis is derived from its heterogeneity. By definition, the type(s) of microbe(s) and primary site of infection are not specified. In other words, any infection associated with organ injury is diagnosed as sepsis. Sepsis occurs as a result of both community-acquired and health care-associated infections. Pneumonia is the most common cause, accounting for about 50% of the cases, followed by intraabdominal and urinary tract infections (11). Staphylococcus aureus and Streptococcus pneumoniae are the most common Gram-positive isolates, whereas Escherichia coli, Klebsiella species, and Pseudomonas aeruginosa are the major...
Gram-negative isolates. The mortality is often higher in extrapulmonary sepsis (12). Understanding a common pathological pathway in sepsis is of great importance to develop potential interventions.

NEUTROPHILS AND ORGAN INJURY IN SEPSIS

Neutrophils consist of about 50 to 70% of all circulating leukocytes and are considered prime immune cells to fight against infection. Upon microbial invasion, inflammatory responses are initiated to recruit neutrophils to the site of infection for host defense. They use phagocytosis, degranulation, and the release of nuclear materials in the form of neutrophil extracellular traps (NETs) for microbial killing (13). When infection is contained, neutrophils can work locally and eradicate microbes. In sepsis, inflammatory responses are rather systemic, and neutrophils can behave as double-edged swords. As much as they help to eradicate microbes, they can damage various organs and contribute to the development of multiple organ injury because they experience impairment of migration to the infection site and instead migrate to the vital organs (14, 15). An untoward binding of neutrophils to the endothelium, NET formation, and subsequent circulatory occlusion cause tissue ischemia and organ injury (14, 16, 17). Lytic factors and proinflammatory cytokines released by neutrophils that infiltrate vital organs also contribute to organ injury.

Considering these profiles, neutrophil behavior needs to be adequately controlled to prevent tissue destruction and harmful sequela during sepsis (18). Understanding how neutrophil behavior is regulated in sepsis is an immense issue. Integrins are heterodimeric molecules consisting of \( \alpha \) and \( \beta \) subunits and are among the most critical adhesion molecules (19). At least 18 \( \alpha \) and 8 \( \beta \) subunits with 24 distinct integrins have thus far been identified in mammals. The various circulating leukocyte subsets display different combinations of integrins, and neutrophils express predominantly \( \beta 2 \) integrins, with small amounts of \( \beta 1 \) and \( \beta 3 \) integrins (18). \( \beta 2 \) integrins are critical molecules among a diverse array of molecules that regulate the function of neutrophils.

Thus, we focus here on reviewing the role of \( \beta 2 \) integrins in neutrophils and sepsis. It is not surprising that there is an interest in using neutrophils as a biomarker for sepsis diagnosis and prognosis due to their paradoxical roles, and one of the \( \beta 2 \) integrin members, \( \alpha X \beta 2 \), has previously attracted attention (20).

ROLE OF \( \beta 2 \) INTEGRINS IN NEUTROPHILS

\( \beta 2 \) integrins are exclusively expressed on leukocytes (also called “leukocyte integrins”) and are paired up with one of the following four \( \alpha \) subunits: \( \alpha L \), \( \alpha M \), \( \alpha X \), or \( \alpha D \). The importance of \( \beta 2 \) integrins in sepsis is undoubtedly appreciated for the disease called leukocyte adhesion deficiency type I (LAD I). This is an autosomal recessive disorder caused by mutations of \( \beta 2 \) integrins that result in defective \( \beta 2 \) integrins or in a deficient level of \( \beta 2 \) integrins (21). The severity of the disease is highly dependent on the expression level of \( \beta 2 \) integrins (22). Patients with <0.5 to 2% \( \beta 2 \) integrin expression are considered to have the severe type and experience recurrent, life-threatening infections, with a mortality rate of about 60% by the age of 2 years unless they receive allogeneic transplantation (22, 23). The most frequent presentation of LAD I is respiratory tract infection (39%), followed by sepsis (29%) and otitis media (27%) (22). Although patients with LAD I show neutrophilia, few neutrophils devoid of functional \( \beta 2 \) integrins are recruited, so little pus formation is noted at the site of infection. In addition, these neutrophils have defects in phagocytosis and bacterial killing (23). Consequently, their ability to eradicate microbes is significantly impaired. \( \beta 2 \) integrins on the cell surface are the primary workforce by binding to their ligands. They are also stored intracellularly and are mobilized to the cell surface upon stimulation. \( \beta 2 \) integrins on the cell surface can shed and are detected in circulating blood in septic patients (24), but the functional role of shed \( \beta 2 \) integrins is not well known.

As illustrated in LAD I, one of the major roles of \( \beta 2 \) integrins is to regulate leukocyte recruitment. In the process of neutrophil recruitment, neutrophils roll over, make a stop (arrest) on the endothelium, and crawl out to reach the site of infection/inflammation.
A classic model of leukocyte recruitment consists of rolling by selectins, adhesion by \( \beta_2 \) integrins, and transmigration at the level of postcapillary venules. However, LAD I also hints at the existence of \( \beta_2 \) integrin-independent neutrophil recruitment. While neutrophil recruitment to the peritoneal cavity was significantly diminished, migration to the lung was observed in an autopsy of a patient with severe type of LAD I (25). As in patients with LAD I, \( \beta_2 \) integrin knockout (KO) mice demonstrate neutrophilia. Given this neutrophilia, \( \beta_2 \) integrin blocking antibody and hematopoietic reconstitution using both wild-type and \( \beta_2 \) integrin KO neutrophils were also used to study the role of \( \beta_2 \) integrin in neutrophil recruitment. A \( \beta_2 \) integrin blocking antibody experiment in preclinical pneumonia models showed that neutrophils were recruited to the lung in both \( \beta_2 \) integrin-dependent and -independent fashions, depending on the microbes/stimuli inducing pneumonia (26). In a study using mice with hematopoietic reconstitution by wild-type and \( \beta_2 \) integrin KO neutrophils, neutrophil recruitment to the lung was dependent on \( \beta_2 \) integrins in Escherichia coli and Pseudomonas aeruginosa pneumonia models, but this was not the case in Streptococcus pneumoniae infection, consistent with the results of a study done with \( \beta_2 \) integrin blocking antibody. So far, it is unclear how different microbial infections are associated with differences in the neutrophil recruitment mechanism. The cecal ligation and puncture (CLP) model is a gold standard preclinical model of extrapulmonary sepsis (3). In this model, neutrophil recruitment to the lung is, in part, dependent on \( \beta_2 \) integrins (27). In sepsis, neutrophils can migrate unintentionally to certain locations, despite the fact that they are not the primary site of infection. Understanding the molecules that regulate neutrophil recruitment to vital organs under various infection conditions is important. When neutrophil recruitment is dependent on \( \beta_2 \) integrins, specifying a member of the \( \beta_2 \) integrins responsible for the recruitment is significant. Understanding the detailed molecular mechanism of neutrophil migration to different tissues and organs during sepsis could open a way to modulate neutrophil behavior.

\( \beta_2 \) integrins are also responsible for a variety of other neutrophil functions. Neutrophil functions include (i) recruitment, (ii) recognition and phagocytosis of microbes, and (iii) microbial killing by production of reactive oxygen species (ROS), release of antimicrobial peptides, and NET formation, and \( \beta_2 \) integrins cover all three of these aspects. Neutrophils are known to live for several hours only. However, their life span could be extended during inflammation, and \( \beta_2 \) integrins also play a role here. Here, we review the role of each \( \beta_2 \) integrin member in neutrophil functions and sepsis. The ultimate goal is to give a consideration of how to modulate \( \beta_2 \) integrins as potential targets in sepsis.

\( \alpha L\beta 2 \), \( \alpha L\beta 2 \) and \( \alpha M\beta 2 \) are the two most abundant \( \beta_2 \) integrins on neutrophils. \( \alpha L\beta 2 \) is one of the most studied integrins and is also called leukocyte function-associated antigen 1 (LFA-1) or CD11a/CD18. This molecule is ubiquitously expressed on all the leukocytes. Intercellular adhesion molecule 1 (ICAM-1), ICAM-2, ICAM-3, ICAM-4, and ICAM-5 are reported as \( \alpha L\beta 2 \) ligands (28–33). While the interaction of \( \alpha L\beta 2 \) with these ligands was studied largely in vitro, the interaction between \( \alpha L\beta 2 \) and ICAM-1 was also validated in vivo (34) (Table 1). ICAM-1 is highly expressed on the endothelial cell, and its interaction with \( \alpha L\beta 2 \) is primarily important for neutrophil arrest. A number of studies have examined the role of this molecule in neutrophil recruitment in sepsis. In a CLP sepsis model using \( \alpha L\beta 2 \) knockout (KO) mice, \( \alpha L\beta 2 \) deficiency worsened survival, which was associated with less neutrophil migration to the abdominal cavity and higher tissue bacterial loads (35). However, neutrophil migration to the spleen, the liver, and the lung was not affected by the absence of \( \alpha L\beta 2 \). Neutrophil recruitment to the lung was rather enhanced without \( \alpha L\beta 2 \). Higher interleukin 6 (IL-6) levels were noted in the lung of \( \alpha L\beta 2 \) KO mice, indicating that they had more lung injury. In line with the results of an \( \alpha L\beta 2 \) KO mice study, the administration of anti-\( \alpha L \) antibody in the CLP sepsis model attenuated the degree of neutrophil recruitment to the peritoneal cavity but did not affect neutrophil migration to the lungs (35). CLP is a model originating from intraperitoneal infection with mixed bacterial flora.
TABLE 1 Interactions between β2 integrins and their ligands and their biological roles in vivo

<table>
<thead>
<tr>
<th>Integrin or ligand</th>
<th>Biological role(s)**</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>αLβ2</td>
<td>In an LPS-induced lung inflammation model, αLβ2–ICAM-1 interaction plays a significant role in neutrophil recruitment to the alveolar space.</td>
<td>Basit et al. (34)</td>
</tr>
<tr>
<td>αMβ2</td>
<td>In a model of TNF-α i.p. injection, leukocyte adhesion to the mesenteric venules was attenuated by anti-αMβ2, anti-αMβ2, and anti-ICAM-1 antibodies. Rolling was attenuated by anti-αMβ2 antibody but not by anti-αLβ2 or by anti-ICAM-1 antibody.</td>
<td>Wolf et al. (50)</td>
</tr>
<tr>
<td></td>
<td>In IL-1β-stimulated venules in the cremaster muscle, neutrophil crawling for transendothelial migration (TEM) was regulated by αMβ2–ICAM-2 interaction.</td>
<td>Halai et al. (48)</td>
</tr>
<tr>
<td></td>
<td>In a mouse model with fibrinogen mutation (N390RLSIGE396), which impaired the interaction with αMβ2, clearance of S. aureus was significantly impaired. No leukocyte recruitment defect was observed.</td>
<td>Flick et al. (57)</td>
</tr>
<tr>
<td>RAGE</td>
<td>In a thioglycolate-induced peritonitis model, blocking of either αMβ2 or RAGE attenuated neutrophil recruitment to the peritoneal cavity. The binding of αMβ2 to RAGE was competed with fibrinogen but not with ICAM-1.</td>
<td>Chavakis et al. (51)</td>
</tr>
<tr>
<td>CD40L</td>
<td>In TNF-α i.p. injection, blocking of the interaction between αMβ2 and CD40L by anti-M7 antibody reduced leukocyte adhesion. In a CLP sepsis model, fewer granulocytes at 20 h were seen with anti-M7 antibody. In addition, enhanced bacterial clearance was observed.</td>
<td>Wolf et al. (50)</td>
</tr>
<tr>
<td>αXβ2</td>
<td>αXβ2 binds to RAGE in vitro. In a thioglycolate-induced peritonitis model, blocking of RAGE attenuated neutrophil recruitment.</td>
<td>Chavakis et al. (51)</td>
</tr>
</tbody>
</table>

**TNF-α, tumor necrosis factor alpha; i.p., intraperitoneal; IL-1β, interleukin 1β.

The importance of αLβ2 to neutrophil recruitment to the peritoneal cavity was also demonstrated in single-strain intraperitoneal infections with Streptococcus pneumoniae and Mycobacterium tuberculosis. αLβ2 KO mice infected with Streptococcus pneumoniae and Mycobacterium tuberculosis also showed worse survival, with higher bacterial loads in tissues (36, 37). In a lipopolysaccharide (LPS) lung instillation model, anti-αL blocking antibody did not affect neutrophil recruitment to the lung as it did in the extrapulmonary sepsis model using CLP (38). Altogether, αLβ2 is significantly responsible for neutrophil recruitment to the peritoneal cavity in the setting of abdominal infection.

αLβ2 is also involved in potentiating neutrophil phagocytosis of certain bacteria. Crosslinking of αLβ2 on neutrophils using the anti-αL antibodies TS1/22 and HI111 facilitated the ingestion of Streptococcus pyogenes, Staphylococcus aureus, or Candida albicans (39). In particular, Streptococcus pyogenes did not get phagocytized without αLβ2 activation, while other species could inject without it. Although crosslinking of αLβ2 using anti-αL antibodies may be an appealing method to facilitate phagocytosis and subsequent microbial killing, it is important to clarify if the αL antibody has blocking ability. For example, TS1/22, which crosslinks with αLβ2, can also block the binding of αLβ2 to its ligand and limit neutrophil migration to the peritoneal cavity. Use of αL antibody with blocking capability should be refrained from in intrabdominal infection. Instead, antibody that crosslinks with αLβ2 without blocking it should be considered under these circumstances.

αMβ2. αMβ2 is also called CD11b/CD18 or complement receptor 3 (CR3) due to its ability to bind to complement iC3b (40). It takes a diverse array of ligands (>40 ligands), which include ICAM-1, fibrinogen, fibronectin, the receptor for advanced glycation end products (RAGE), endothelial protein C receptor, and CD40L (41, 42). A large number of reported ligands were tested only in vitro. Some were also tested in vivo (Table 1). αMβ2 is involved in a wide variety of neutrophil functions, which include recruitment, phagocytosis, ROS formation, NETs, apoptosis, and cytokine production. Whether αMβ2 governs individual neutrophil functions through its interaction with different set of ligands or with the same ligands is an important question.

αMβ2 is involved in neutrophil recruitment, similarly to the involvement of αLβ2 in sepsis. Recruitment to the liver was attenuated in moderate to severe sepsis induced by CLP surgery (43). In contrast, αLβ2 deficiency did not affect neutrophil recruitment to
the liver. In this model, αMβ2 deficiency did not attenuate neutrophil recruitment to the lung (44). Rather, neutrophil recruitment to the peritoneal cavity was increased. This increase was presumably due to a reduction in neutrophil apoptosis by lack of αMβ2, as described in one report (45). In an LPS lung instillation model, anti-αM blocking antibody attenuated neutrophil recruitment to the lung (38), which was different from the effect of anti-αL blocking antibody. Then, is the difference in neutrophil recruitment between αLβ2 and αMβ2 dictated by target organs? Both interact with ICAM-1 for neutrophil recruitment. However, the arrest of neutrophils on ICAM-1 on the endothelium via αLβ2 overshadows the contribution from αMβ2 (46). Neutrophil extravasation in tumor necrosis factor alpha (TNF-α) stimulated vasculature at the cremaster muscle and was significantly attenuated in αLβ2 KO mice, as αLβ2 is highly responsible for adhesion. However, neutrophil extravasation was increased in αMβ2 KO mice. αMβ2 is involved in intraluminal crawling (47), and ICAM-2, in addition to ICAM-1, is an important ligand for αMβ2 (48). In fact, αMβ2 might serve as a brake after the neutrophil migrates beneath the endothelium, as it moves through the interstitial space, and loss of this brake allows neutrophils to extravasate more (46). This, in addition to apoptosis, may also explain enhanced neutrophil recruitment to the peritoneal cavity in αMβ2 KO mice in the CLP model. Neutrophil rolling, arrest, adhesion, crawling, and transendothelial migration were extensively studied using the cremaster muscle circulation (49), and this circulation pattern is applicable to many tissues, including that in the peritoneal cavity. Neutrophil interaction for this circulatory structure almost exclusively takes place at the postcapillary venules. Target organs are also important to determine the mechanism of neutrophil recruitment. For other circulatory structures, the role of αLβ2 and αMβ2 has to be understood separately. In the lung, neutrophil recruitment occurs mainly at the capillaries. The diameter of capillaries in the lung is smaller than that of neutrophils, which makes rolling unnecessary (49). In the liver, the majority of recruitment occurs at sinusoids, and some occurs at the postcapillary. To make things complicated, αMβ2 also binds to the receptor for advanced glycation end products (RAGE) and to CD40L for neutrophil recruitment (50, 51). Thus, the distribution of ligands is also an important consideration.

Phagocytosis is largely classified as either complement-mediated or Fc receptor (FcR)-mediated phagocytosis. αMβ2 directly binds to iC3b for complement-mediated phagocytosis. In a CLP model using αMβ2 KO mice, αMβ2 deficiency worsened the outcome of sepsis with impaired phagocytosis (44). Because phagocytosis in the CLP model is largely complement dependent (52), this result is compatible with the fact that αMβ2 is a phagocytosis receptor for iC3b-coated microbes. αMβ2 is also involved in FcR-mediated phagocytosis (53–55). This is driven by the cross talk between FcR and αMβ2. Among a number of FcRs, the role of FcRγ in phagocytosis was studied in sepsis, and its deficiency was found to improve bacterial phagocytosis. Because FcRs are divided into activating and inhibitory FcRs (56), other FcRs and their relevance to αMβ2 need to be studied in vivo in the future. Fibrinogen is a ligand for αMβ2, and αMβ2-fibrinogen interaction was shown to be important for Staphylococcus aureus clearance in vivo (57). Fibrinogen inhibits complement-mediated opsonization on microbes (58) and can negatively impact complement-mediated phagocytosis. In contrast, the binding of fibrinogen to IgG enhances IgG-mediated phagocytosis (59). αMβ2-fibrinogen interaction may be an additional mechanism to augment FcR-mediated phagocytosis through αMβ2.

NET formation is one form of cell death. NET formation plays an important role in organ injury in sepsis, as DNase I treatment against NETs improved sepsis survival in a preclinical sepsis model (60). The involvement of αMβ2 has been shown in immune complex-induced NETs, as well as in ventilator-induced lung injury (61, 62). In these two previous studies, the formation of NETs was reduced by anti-αM blocking antibody, not by anti-αL blocking antibody. Although NET formation is critical in sepsis pathophysiology, there is no study specifically examining the involvement of αMβ2 in this setting. Because αMβ2 deficiency worsens the outcome of sepsis, however, simply administering anti-αM blocking antibody would not be a good option. Identification of a ligand
for αMβ2 to initiate NET formation would be beneficial to attenuate it but preserve phagocytosis, for example, for therapeutic intervention. Apoptosis is another form of cell death. Apoptosis is considered immunologically silent compared to other types of cell death such as necrosis, but it is important to note that antiapoptotic therapy improved the survival of sepsis cases (63). In studies using spleen samples from septic patients and preclinical models, a wide variety of leukocytes undergo apoptosis (63, 64). One anesthetic, sevoflurane, attenuated neutrophil apoptosis and improved survival in a CLP model (65). The effect of αMβ2 on apoptosis is context dependent and requires further investigation for a therapeutic approach. αMβ2 engagement to ICAM-1 and fibrinogen attenuates neutrophil apoptosis (66). In contrast, phagocytosis mediated by αMβ2 accelerates apoptosis.

Proinflammatory cytokine production is an important physiological response in infection. High levels of circulating proinflammatory cytokines are associated with circulatory impairment. In addition, TNF-α serves as a proapoptotic signal. In a CLP model, αMβ2 knockout mice showed higher TNF-α and IL-1β levels than wild-type mice (44). αMβ2 has a regulatory role on Toll-like receptor 4 (TLR4) function, and αMβ2 deficiency exaggerates TLR-mediated responses (67). Although it is not known if a specific ligand is involved in αMβ2-TLR4 cross talk, activating αMβ2 would be important to reduce exaggerated proinflammatory responses.

αXβ2. αXβ2 is also called CD11c/CD18 or complement receptor 4 (CR4). Classically, this molecule has been used as a marker for dendritic cells. However, its expression has been shown in other type of leukocytes, including neutrophils. The expression of αXβ2 on neutrophil cell surfaces in patients showed high sensitivity and specificity to differentiate systemic inflammatory response syndrome (SIRS) with or without infection, indicating that αXβ2 may serve as a diagnostic marker for sepsis (68). αXβ2 is highly homologous to αMβ2, and they share a number of ligands, including ICAM-1, iC3b, fibrinogen, and RAGE. The majority of studies were performed in vitro, and the biological roles of αXβ2 and its diverse array of ligands are yet to be determined in vivo. The involvement of RAGE-αXβ2 interaction in neutrophil recruitment has been described in a thioglycolate-induced peritonitis model (51) (Table 1). RAGE is highly expressed on the endothelium, and interaction with its receptors contributes to sepsis pathophysiology (69).

αXβ2 is also classified as a complement receptor, and its involvement in phagocytosis has been shown in vitro (70). How this molecule is involved in phagocytosis in sepsis needs to be studied. Notably, αXβ2 directly recognizes Candida albicans for its eradication (71).

αDβ2. αDβ2 (CD11d/CD18) is a rather understudied β2 integrin member. αDβ2 is highly homologous to αMβ2 and αXβ2 and binds to ICAM-1, ICAM-3, and vascular cell adhesion molecule 1 (VCAM-1) (72, 73). Its expression on neutrophils has been seen in patients with lung injury (74). In a model using intraperitoneal injection of malaria, survival was improved and less lung injury was observed in αDβ2 knockout mice (75). However, in intraperitoneal injection of Salmonella enterica serovar Typhimurium, survival was decreased, with more pyroptosis in αDβ2 knockout mice (76). The role of αDβ2 in sepsis needs to be investigated in detail in the future to decipher how αDβ2 is involved in different microbial infections.

**POTENTIAL THERAPY AGAINST β2 INTEGRINS FOR SEPSIS**

Clearly, each β2 integrin member plays a significant role in a number of neutrophil functions by interacting with redundant ligands. Neutrophils can be both beneficial and detrimental in sepsis, and blocking β2 integrins in general will clearly recapitulate LAD I by inhibiting the beneficial part of neutrophil functions, even though NETs may be attenuated. αLβ2 and αMβ2 KO mice showed worse outcomes than wild-type mice, with defects in recruitment and phagocytosis by phagocytes, respectively (35, 44). Although we do not know what blockade of αXβ2 and αDβ2 shows at this point, simply inhibiting each β2 integrin member may not be appropriate to improve sepsis outcome. For example, improving neutrophil recruitment to the site of infection and
subsequent phagocytosis while reducing apoptosis and NET formation in organs can be considered as a direction for integrin-based therapy.

An interesting example is anti-M7 antibody treatment to block the interaction between αMβ2 and CD40L (50). CD40L is stored in α granules in unstimulated platelets but is rapidly translocated to their surface when activated, where it is cleaved and released into circulation as soluble CD40L. Circulating soluble CD40L levels are increased in septic patients and are independently associated with their mortality (77). In addition, CD40L is expressed on inflamed endothelial cells (50, 78). Anti-M7 antibody blocked the interaction of αMβ2 with CD40L, but not that with other αMβ2 ligands, namely, ICAM-1, fibrinogen, RAGE, vitronectin, and heparin (42). This antibody attenuated neutrophil recruitment to the peritoneal cavity in the severe CLP model. Despite less recruitment to the peritoneal cavity, the administration of this antibody lessened bacterial loads in tissues. Anti-M7 antibody increased the binding of αMβ2 to IC3b and promoted phagocytosis. Furthermore, anti-M7 antibody did not alter proinflammatory cytokine levels, suggesting that this did not affect αMβ2 Toll-like receptor 4 cross talk. Administration of anti-M7 antibody improved overall sepsis survival. However, it was not effective when given at 2 h after CLP, indicating that αMβ2-CD40L interaction occurred at an earlier time in the course of sepsis. A number of known ligands bind to β2 integrins at the top domain of the α subunit, called the I domain. CD40L binds to the I domain of αMβ2. It is not clear how anti-M7 antibody augments IC3b binding. Understanding the biological role of individual ligands and their binding sites would bring insight in the future.

Another example is the use of small-molecule leukoadherin-1 (LA-1). LA-1 also binds to the I domain of αMβ2 and serves as an αMβ2 agonist. Administration of this compound seems to keep αMβ2 constitutively active and increases the binding of αMβ2 to ICAM-1, IC3b, and fibrinogen (79). Cell migration requires cycles of integrin activation and deactivation (80, 81). Supporting this idea, experiments using knock-in mice expressing activating mutants of αLβ2 showed that leukocytes increased adhesion and decreased migration (82). In line with this, LA-1 impaired phagocyte migration to the peritoneal cavity in the CLP model. However, LA-1 enhanced phagocytosis (79). The administration of LA-1 in the CLP model reduced proinflammatory cytokine levels and lessened bacterial loads in tissues, with survival benefits (83). LA-1 attenuated TLR4 function by increasing αMβ2-TLR4 (84), which could explain the lower proinflammatory cytokine levels in the LA-1 arm. While anti-M7 antibody affected the interaction between a subset of ligands and αMβ2, it would be worthwhile to study the effect of LA-1 on αMβ2 function in general, including in cell death. Whether the administration of LA-1 after the onset of sepsis has a beneficial effect in sepsis cases also needs to be determined.

Anti-M7 antibody and LA-1 are examples of modulating a certain aspect of αMβ2 for benefit in sepsis. Understanding the biological role of each β2 integrin member is critical and will serve as a foundation for future therapeutic approaches.

Conclusion. Although β2 integrins have been known for more than 3 decades, their biological roles still need to be investigated. Application of this knowledge to sepsis therapy requires the identification of β2 integrin-ligand combinations appropriate for intervention because each integrin has redundant function and overlapping ligands.

ACKNOWLEDGMENTS

This work was in part supported by the CHMC Anesthesia Foundation (K.Y.) and by NIH grant R01GM118277 (K.Y.). We have no conflicts of interest to declare.

REFERENCES


Minireview Infection and Immunity


Jimenez-Alcazar M, Rangaswamy C, Panda R, Bitterling J, Simsek YJ, Long


Koichi Yuki, M.D., received his M.D. from the University of Tokyo. He did clinical training at the University of Hawaii, at Massachusetts General Hospital, and at Boston Children’s Hospital, and did postdoctoral research training on β2 integrins under Dr. Motomu Shimaoka and Dr. Timothy Springer at the Immune Disease Institute. Currently, he is an Associate Professor of Anaesthesia at Harvard Medical School and also works as a pediatric cardiac anesthesiologist at Boston Children’s Hospital. He has been interested in organ injury driven by neutrophils and has been working on β2 integrins and sepsis for more than 10 years.

Lifei Hou, Ph.D., received his Ph.D. in Pharmacology from the Chinese Academy of Sciences. He did postdoctoral training at the Immune Disease Institute and Boston Children’s Hospital under Dr. Eileen Remold-O’Donnell and Dr. Koichi Yuki. Before arriving in the United States, he was an Assistant Professor at the Shanghai Institute of Materia Medica, Chinese Academy of Sciences. He currently holds the position of Instructor in Anaesthesia, Harvard Medical School, and is a Research Associate at the Department of Anesthesiology, Critical Care and Pain Medicine, Boston Children’s Hospital. He has studied inflammation and immune disorders for more than 15 years.