Cell adhesion molecules in the pathogenesis of and host defence against microbial infection

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Abstract

Eukaryotic cell adhesion molecules (CAMs) are used by various cells and extracellular molecules in host defence against infection. They are involved in many processes including recognition by circulating phagocytes of a site of inflammation, transmigration through the endothelial barrier, diapedesis through basement membrane and extracellular matrix, and release of effector mechanisms at the infected site. CAMs involved in leucocyte–endothelial cell interaction include the selectins, integrins, and members of the immunoglobulin superfamily. However, CAMs are also used by various microorganisms (protozoa, fungi, bacteria, and viruses) during their pathogenesis. For example, bacteria that utilise CAMs include Mycobacterium tuberculosis, Listeria monocytogenes, keratitis spp, enteropathogenic Escherichia coli, Shigella spp, Neisseria spp, Bordetella spp, and Borrelia burgdorferi. In addition, CAMs are involved in the pathogenetic effects of the RTX toxins of Pasteurella haemolytica, Actinobacillus actinomycetemcomitans, and the superantigen exotoxins of Staphylococcus aureus and Streptococcus pyogenes. A recurrent and topical theme of potential importance within the bacterial group is the intimate relation between CAMs, bacterial protein receptors, and type III secretion systems. For example, the IpaBCD protein complex is secreted by the type III system of Shigella flexneri and interacts with αβ integrin on the eukaryotic cell surface, followed by Rho mediated internalisation; this illustrates the relevance of cellular microbiology. CAMs might prove to be novel therapeutic targets. Comparative genomics has provided the knowledge of shared virulence determinants among diverse bacterial genera, and will continue to deepen our understanding of microbial pathogenesis, particularly in the context of the interaction of prokaryotic and eukaryotic molecules.

Keywords: cell adhesion molecules; microbial infection; host defence

The environment of any cell is a complex mixture of soluble molecules in the extracellular matrix (ECM), other cells, and insoluble molecules that form tissue matrices. Cell adhesion molecules (CAMs) are the means by which a cell communicates with other cells and the ECM. Thus, the cell samples its environment by means of complex arrays of CAMs present on the cell surface and integrated into the cell membrane. Although named “cell adhesion molecules” because they mediate cellular attachment to other cells and the ECM, this “stickiness” is not always the prime function of a particular molecule. For example, cellular attachment is the means by which a cell maintains itself in a certain relative location, to allow the cell to sample its environment and respond to molecules that are part of neighbouring cells and insoluble matrices.1

All CAMs are transmembrane glycoproteins and have extracellular, transmembrane, and intracellular domains; thus, they connect the interior and exterior of a cell. All molecules bind their specific receptor or ligand, and this binding leads to a conformational change in the extracellular domain. All CAMs are attached to an intracellular molecule, which affects the function of the cell through the “second messenger pathway”, producing changes in the cytoskeleton or the chemical composition of the cell. There are six families of CAMs; the immunoglobulin-like superfamily, the cadherins, the integrins, the receptor protein tyrosine phosphatases, the selectins, and the hyaluronate receptors. For information on each of their features, ligands and functions see the review by Freemont.2 CAMs can modulate disease processes, whether they are expressed normally or not.3 The role of CAMs or their ligands in abnormal processes occurs either as part of a physiological response to a pathological event—for example, inflammation or infection—or as part of the pathological process itself.4

Microorganisms have developed various strategies to subvert host defences and permit their particular lifestyle, among which several general themes have emerged. To enable succinct and meaningful documentation of particular microbial–CAM interactions under the headings below, I will first outline some general concepts and their relation to microbial pathogenesis.

Current and relevant topics in microbial pathogenesis

GRAM NEGATIVE BACTERIAL SECRETION SYSTEMS

Four types of secretion in Gram negative bacteria have been documented: types I, II, III, and IV.1 Type I is sec dependent and is exemplified by the secretion of α-haemolysin by Escherichia coli; it secretes effector proteins across the inner membrane, periplasm, and outer membrane in a one step process. Type II secretion is sec dependent and secretes effector
proteins across the inner membrane into the periplasm using the general secretory pathway (GSP)\(^\text{4}\); in the periplasm, the C-terminal sequence is removed and the protein is then secreted across the outer membrane by a complex of 12 or more proteins; known as the terminal branch of the GSP. The best studied example of type II secretion is the pullulanase (pulA) enzyme of *Klebsiella oxytoca*.\(^\text{4}\) Type III secretion occurs on host cell contact, and facilitates the translocation of effector molecules directly into the host cell cytoplasm. Type III systems are thought to be direct descendants of the flagellar assembly and consist of 20 or more proteins, which span the cell envelope. Type III systems are crucial for virulence in various organisms including *Salmonella* spp, *Shigella* spp, enteropathogenic *E coli* (EPEC), and *Bordetella* spp.\(^\text{5}\) Effector proteins are targeted for type III secretion by the N-terminal sequence of their encoding mRNA molecule.\(^\text{6}\) Type IV secretion occurs in various species and is responsible for secretion of—for example, *Bordetella pertussis* P.69 pertactin and the serum resistance protein, brkA. Transport across the inner membrane, periplasm, and outer membrane probably occurs in three separate stages with N-terminal and C-terminal processing of the transported protein.\(^\text{6}\)

### CELLULAR MICROBIOLOGY

Microorganisms depend on their ability to subvert and subjugate eukaryotic host cells for their pathogenesis, and knowledge of eukaryotic cell physiology is becoming increasingly relevant to understanding microbial pathogenesis.\(^\text{7}\) Examples include viral adhesion/invasion and bacterial type III secretion systems, which facilitate bacterial internalisation and persistence. Pedestal formation in EPEC infection and phagocytosis are both mediated by cellular GTPases (Rho, Rac, and Cdc42), which are molecular switches for control of signal transduction pathways, linking membrane receptors to mitogen activated protein kinase and the signal transduction pathways, linking membrane receptors to mitogen activated protein kinase and the actin cytoskeleton in various cell types. RTX (repeats in toxin) toxins—for example, *E coli* α-haemolysin (hlyA), bind to lymphocyte function associated antigen 1 (LFA-1), a \(\beta\) integrin (CD11a/CD18),\(^\text{8}\) causing pore formation and cytolysis. Several studies suggest that RTX toxins kill target cells by apoptosis.

Host defences are frequently responsible for disease manifestations, either directly in the case of the early response of plants to plant pathogens, or indirectly in the case of the utilisation of a particular pathway of phagocytosis that lacks a respiratory burst and enables intracellular survival in humans. Phagocytosis is an important host defence and in macrophages it is mediated by at least two receptors: complement receptor 3 (CR3), a \(\beta\) integrin, and the immunoglobin receptor (Fc\(\gamma\)R). CR3 consists of a 165 kDa \(\alpha\) subunit (CD11b) and an 85 kDa \(\beta\) subunit (CD18). Rho, Rac, and Cdc42 are GTPases that act as molecular switches in the reorganisation of the actin cytoskeleton, and the profile of their involvement depends on the particular phagocytic mechanism.\(^\text{9}\) Immunoglobulin dependent uptake (type I phagocytosis) requires Cdc42 and Rac and involves pseudopod extension, membrane ruffling, a respiratory burst, and an inflammatory response. CR3 dependent uptake (type II phagocytosis) is mediated by CR3, requires only Rho, and occurs without a respiratory burst.\(^\text{10,11}\) Differential GTPase involvement between the two types of phagocytosis probably determines the physiological differences between Fc\(\gamma\)R and CR3 mediated phagocytosis.\(^\text{12,13}\)

The purpose of this review is to document the interaction of eukaryotic CAMs and microorganisms in the context of: (1) the microbe in its strategy to gain access to and cause disease in the host, and (2) the host in its strategy to defend itself against infection. These aspects will be dealt with in this order.

### Microbial pathogenesis facilitated by CAMs

In general, the interaction of microbes with eukaryotic CAMs can be divided arbitrarily into those interactions mediated by microbial molecules that remain associated with the prokaryotic or eukaryotic cell envelope, and those mediated by secreted microbial products and toxins.

#### PROTOZOA

Table 1 summarises the interactions of eukaryotic CAMs with protozoa. Erythrocytes infected with the malaria parasite, *Plasmodium falciparum*, sequester in deep tissues by binding to endothelial cells. This favours parasite

<table>
<thead>
<tr>
<th>Protozoan/fungus</th>
<th>Microbial molecules</th>
<th>Eukaryotic CAM</th>
<th>Role in pathogenesis</th>
<th>Ref</th>
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<tr>
<td><em>Plasmodium falciparum</em></td>
<td>PfEMP-1, erythrocyte band 3 related adhesin, sequestrin, and break point open reading frame</td>
<td>Endothelial CD36, ICAM-1, ELAM-1, VCAM-1</td>
<td>Sequestration of infected RBCs in deep tissues by endothelial cell binding; probably responsible for cerebral malaria</td>
<td>15–18</td>
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<tr>
<td><em>Leishmania major</em></td>
<td>—</td>
<td>CR3 ((\alpha) integrin)</td>
<td>Mediates CR3 phagocytosis with reduced or absent respiratory burst</td>
<td>19</td>
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<td><em>Entamoeba histolytica</em></td>
<td>170 kDa Gal/GalNAc lectin</td>
<td>—</td>
<td>Lectin similar to (\beta) integrin and probably regulates integrin expression</td>
<td>20</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>INT1 ((\alpha_2/\alpha_3) integrin-like)</td>
<td>—</td>
<td>Int1 has homology to human integrins and is crucial for adhesion, hyphal growth, and pathogenicity</td>
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<tr>
<td><em>Candida tropicalis</em></td>
<td>(\beta_1), Integrin-like protein</td>
<td>CR3 ((\alpha), (\beta) integrin)</td>
<td>Adhesion</td>
<td>23</td>
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<tr>
<td><em>Histoplasma capsulatum</em></td>
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<td>CR3 ((\alpha), (\beta) integrin)</td>
<td>CR3 phagocytosis</td>
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<tr>
<td><em>Pneumocystis carinii</em></td>
<td>Gp120 (integrin-like protein)</td>
<td>CR3 ((\alpha), (\beta) integrin)</td>
<td>CR3 phagocytosis</td>
<td>25, 26</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>(\alpha) Integrin subunit</td>
<td>Interaction of gp120 with A549 cells upregulates host cell (\alpha), integrin expression</td>
<td>27</td>
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</tbody>
</table>

CR3, complement receptor 3; ELAM-1, endothelial leucocyte adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1; PfEMP-1, *Plasmodium falciparum* erythrocyte membrane protein 1; VCAM-1, vascular cell adhesion molecule 1.
growth, evasion of host defences, and occlusion of the microcirculation leading to hypoxia and lactic acidosis, which together are probably responsible for cerebral malaria. Several CAMs on endothelial and other cells mediate the interaction with infected erythrocytes, including CD36, intercellular cell adhesion molecule 1 (ICAM-1), endothelial leucocyte adhesion molecule 1 (ELAM-1), and vascular cell adhesion molecule 1 (VCAM-1).

Leishmania major, the cause of leishmaniasis, is dependent for its pathogenesis on an intracellular lifestyle in the macrophage. The promastigote binds to CR3 and is phagocytosed by a type II pathway.

Entamoeba histolytica kills host cells by adhering via a 170 kDa Gal/GalNAc lectin on its surface. This molecule contains a cytoplasmic domain with similarity to the β1 integrin motif, a crucial regulator of integrin activities in other systems mediated by inside out signalling.

**Fungi**

Table 1 summarises interactions of eukaryotic CAMs with fungi. Fibronectin receptors consisting of integrin-like proteins have been identified in *Candida albicans* and *Candida tropicalis* using various approaches, including monoclonal and polyclonal antibodies to αβ integrins. The *C. albicans INT1* gene encodes a surface protein that has 18% amino acid identity with the ligand binding (I) domain of the human α5β integrin, and is necessary for adhesion, filamentous growth, and virulence. However, proteins other than integrins may be involved because RGD motif (Arg-Gly-Asp) deletion in recombinant fibronectin does not decrease adherence. Yeasts also bind CR3 and are thus phagocytosed by macrophages by a type II pathway.

**Bacteria**

Table 2 summarises interactions of eukaryotic CAMs with bacteria. *Staphylococcus aureus* and *Streptococcus pyogenes* produce various proteins of the superantigen family that bind specifically to the Vβ chains of the T cell receptor with high affinity, activating larger numbers of T cells than processed antigens and resulting in massive cytokine release. Several clinical syndromes have been linked with superantigens such as food poisoning, toxic shock syndrome, and Kawasaki syndrome. Bacterial superantigens implicated in human disease include toxic shock syndrome toxin 1 (TSST-1), staphyloccocal enterotoxin A (SEA), staphyloccocal enterotoxin B (SEB), staphyloccocal enterotoxin C (SEC), and streptococcal pyrogenic exotoxin A (SpeA). Released cytokines upregulate vascular and neutrophil adhesion molecules, leading to neutrophil influx. Leucocyte recruitment occurs through sequential involvement of different CAM families: selectins (E, P, and L), integrins (αβ, β1, β2), and members of the immunoglobulin superfamily (ICAM-1, ICAM-2, and VCAM). *Streptococcus pyogenes* pyrogenic exotoxin B (SpeB) is a crucial virulence factor for invasive disease. A variant of SpeB, SpeB2, produced by all isolates of the virulent M1 serotype, contains an RGD sequence that mediates binding to αβ or α5β integrins, integrins on endothelial cells and platelets, respectively.

Mycobacterium tuberculosis is a facultative intracellular pathogen that enters alveolar macrophages, resists macrophage killing mechanisms, establishes a chronic infection, and disseminates via the blood and lymph, frequently in the absence of symptoms. The organism may bind to a number of receptors including the integrins, CR3, and CR4. CR3 binding leads to CR3 phagocytosis with an absent respiratory burst. Binding of macrophage CR3 also reduces interleukin 12 production, with a consequent suppression of macrophage responses and T helper type 1 (Th1) dependent cell mediated immunity. On the other hand, CR4 binding results in phagocytosis with a respiratory burst. In addition, *M. tuberculosis* chaperonin 60 (heat shock protein 65) upregulates E selectin and ICAM-1 on vascular endothelium, and is thus proinflammatory and immunomodulatory, with the potential to induce tissue pathology.

Listeria monocytogenes is a Gram positive bacillus capable of intracellular replication in enterocytes, fibroblasts, dendritic cells, and macrophages. The normal route of infection is via the intestinal epithelial M cells of the Peyer's patches, followed by basolateral invasion of enterocytes, and dissemination by blood and lymphatics to other tissues. Bacterial internalin A (InlA), a cell wall associated protein, binds E-cadherin on epithelial cells and is essential for internalisation. Internalin B (InlB) is required for entry into hepatocytes but its receptor is unknown. InlA and InlB contain leucine rich repeats and are members of a superfamly of leucine rich repeat containing proteins that are found only in pathogenic bacteria—for example, *Shigella flexneri* ipaH.
Table 2  Bacteria that interact with eukaryotic cell adhesion molecules (CAMs) during their pathogenesis

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Bacterial molecule</th>
<th>Eukaryotic CAM</th>
<th>Role in pathogenesis</th>
<th>Refs</th>
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<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>TSST-1, SEA, SEB, SEC</td>
<td>P, E, L selectins, αββ, ββ integrins, ICAM-1, 2, VCAM</td>
<td>Mitogenic with massive cytokine release</td>
<td>32–35</td>
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<tr>
<td>Streptococcus pyogenes</td>
<td>SpeA</td>
<td>P, E, L selectins, αββ, ββ integrins, ICAM-1, 2, VCAM</td>
<td>Mitogenic with massive cytokine release</td>
<td>31</td>
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<tr>
<td>Mycobacterium tuberculosis</td>
<td></td>
<td></td>
<td>RGD mediated adhesion to endothelium and platelets</td>
<td>36</td>
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<tr>
<td>Listeria monocytogenes</td>
<td>Internalin A</td>
<td></td>
<td>Invasion of epithelial (including M) cells</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Internalin B</td>
<td></td>
<td>Invasion of epithelial (including M) cells and hepatocytes</td>
<td>41</td>
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<tr>
<td>Escherichia coli</td>
<td>Antigen 43</td>
<td>Integrins</td>
<td>RGD motif; secreted by type IV pathway</td>
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<tr>
<td>Enteropathogenic E. coli (EPEC)</td>
<td>Intimin</td>
<td>Integrins</td>
<td>Adherence, pore formation, and cytosis</td>
<td>8</td>
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<tr>
<td>Enterococcus faecalis</td>
<td></td>
<td></td>
<td>Adherence and pathogenesis of A/E lesion</td>
<td>43–45</td>
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<tr>
<td>Staphylococcus aureus</td>
<td>FimD</td>
<td>Integrins</td>
<td>Bryant translocation into M cells</td>
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<td>Neisseria spp.</td>
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<td></td>
<td>Adherence and phagocytosis</td>
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<tr>
<td>Bordetella pertussis</td>
<td>fimD</td>
<td>Integrin VLA-5</td>
<td>Adhesion</td>
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<tr>
<td>Bordetella bronchiseptica</td>
<td>P.70</td>
<td>CR3 integrin</td>
<td>CR3 upregulation as a result of selectin</td>
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<td>Bartonella bacilliformis</td>
<td>P.68</td>
<td>Integrins</td>
<td>CR3 integrin, Mac-1 integrin receptor (CD11b/CD18)</td>
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<tr>
<td>Pasteurella haemolytica</td>
<td>Leucotoxin</td>
<td>ββ integrin</td>
<td>CR3 integrin, Mac-1 integrin receptor (CD11b/CD18)</td>
<td>63</td>
</tr>
<tr>
<td>Borrelia burgdorferi</td>
<td>CR3 integrin, αββ integrin</td>
<td>PC66 pertactin</td>
<td>RTX toxin binding, pore formation, and apoptosis</td>
<td>70</td>
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<tr>
<td>Propionibacterium acnes</td>
<td>LPS</td>
<td>Upregulation of E and P selectins</td>
<td>Adhesion and invasion via CR3 phagocytosis</td>
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<tr>
<td>Actinobacillus</td>
<td>Leucotoxin</td>
<td>ββ integrin</td>
<td>Adhesion and invasion via CR3 phagocytosis</td>
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<td>Mycoplasma penetrans</td>
<td>Membrane lipoprotein</td>
<td>CD18 (β subunit of β integrins)</td>
<td>Mitogenicity</td>
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</table>

A/E attaching and effacing; CHO, Chinese hamster ovary; CR3, complement receptor 3; FHA, filamentous haemagglutinin; fimD, minor fimbrial subunit D; hsp65, heat shock protein 65; HSPG, heparan sulphate proteoglycan; ICAM-1, intercellular adhesion molecule 1; LFA, lymphocyte function associated antigen 1; LPS, lipopolysaccharide; PT, pertussis toxin; SEA, staphylococcal enterotoxin A; SpeA, streptococcal pyrogenic exotoxin A; TcRA, tracheal colonisation factor A; Tir, translocated intimin receptor; TSST-1, toxic shock syndrome toxin 1; VCAM-1, vascular cell adhesion molecule.

**Yersinia pestis** yopM, *B. pertussis* filamentous haemagglutinin (FHA), and *Treponeema pallidum* TpLPRR.77–79 The leucine rich repeat region of InlA mediates binding to caco-2 cells.81

*Escherichia coli* hlyA is an RTX toxin that kills human immune cells. It is secreted by a type I pathway and is localised to the surface of lymphocytes by binding to LFA-1, a ββ integrin (CD11b/CD18). EPEC is the prototype of a family of pathogens that causes “attaching and effacing” lesions, characterised by intimate adherence between bacteria and epithelial cells and effacement of intestinal microvilli.70 Several proteins, designated EspS, translocated by a type III system, are essential for this activity. Adherence was shown to occur as a result of the binding of a bacterial intimin protein to a “eukaryotic” receptor. However, it has been discovered recently that the host receptor is in fact a bacterial product whose transfer into host cells is dependent on the type III system and two other proteins (espA and espB), and it has been renamed Tir (translocated intimin receptor) or EspE. In addition, ββ integrin may act as an alternative receptor.38–40

*Shigella flexneri*, the cause of bacillary dysentery, enters eukaryotic cells by reorganising the actin cytoskeleton, a process requiring the Ipa proteins secreted by the type III system.48 The shigellosis IpaBCD complex has been shown to bind αββ integrin, an interaction that appears to play an active role during entry.49–51 Because ββ integrins can associate with the actin cytoskeleton through the C-terminus, this may mediate invasion. The fact that Rho, a small GTPase involved in focal adhesion formation, is required for this is consistent with this hypothesis.52

*Y. pseudotuberculosis* and *Y. enterocolitica* cause food poisoning and, after ingestion, translocate across the intestinal epithelial barrier via the M cells of Peyer’s patches. M cells are a major site of invasion of bacteria at least during the early stages of infection. In the mouse, M cells are invaded by both *Y. pseudotuberculosis* and *Y. enterocolitica*.53, 54 *Y. pseudotuberculosis* invasin protein attaches to ββ integrins on M cells, leading to invasion.55–57 Murine Peyer’s patch M cells contain ββ integrins but enterocytes do not. Internalisation requires high affinity binding and for adhesion kinase activity.58 Invasin also promotes binding to and activation of B lymphocytes via ββ integrin.59

*Legionella pneumophila* is an aerobic Gram negative bacillus, which causes Legionnaire’s
disease in predisposed individuals. The organism has an intracellular lifestyle and replicates in alveolar macrophages after opsonin dependent, CR3 mediated phagocytosis mediated by actin polymerisation at the site of entry, which is dependent on tyrosine phosphorylation of various host proteins.

Neisseria gonorrhoeae and Neisseria meningitidis colonise the human host, and this can either be asymptomatic or lead to the severe local or systemic inflammatory responses that characterise gonorrhoea and meningococcal meningitis, respectively. Primary attachment is mediated by bacterial pili, with a more intimate attachment mediated by the bacterial opacity associated (Opa) proteins, originally identified because their expression results in a change of colony opacity and colour as a result of bacterial aggregation. Opa proteins utilise heparan sulphate proteoglycan (HSPG) in Chang conjunctiva cells for intimate adhesion and integrin mimicry: however, the mechanism of entry is still poorly understood. Reproduced from Dehio et al, with the permission of Elsevier Science, Oxford, UK.

Figure 1 Mechanisms of Opa50 mediated internalisation into different epithelial cell lines. (a) In Chang conjunctiva epithelial cells, heparan sulphate proteoglycan (HSPG) dependent internalisation involves the activation of phosphatidylinositol dependent phospholipase C (PC-PLC), which results in the generation of the second messenger diacylglycerol (DAG) from phosphatidylinositol (PC). DAG activates the acidic sphingomyelinase (ASM), which then generates ceramide from sphingomyelin. By an unknown process, ceramide is implicated in mediating cytoskeletal reorganisation and bacterial uptake by a mechanism that resembles conventional phagocytosis. (b) Efficient bacterial uptake into HeLa cervical carcinoma cells and Chinese hamster ovary (CHO) cells also relies on the ability of Opa50 to mediate binding to the extracellular matrix protein, vitronectin (VN), and thereby to co-ligate HSPG and αvβ5 integrin vitronectin (VN) receptors, including αvβ5. This internalisation process appears to be dependent on the activity of protein kinase C (PKC). (c) In HEp-2 larynx carcinoma cells, efficient bacterial uptake of Opa50 expressing gonococci requires binding of the extracellular matrix protein, fibronectin (FN), which results in a co-ligation of HSPG and the FN receptor αvβ3 integrin: however, the mechanism of entry is still poorly understood. Reproduced from Dehio et al, with the permission of Elsevier Science, Oxford, UK.

pathogenesis. The minor fimbrial subunit, fimD, has been shown to bind to integrin VLA-5 (very large antigen 5) and heparin, an interaction that is important for colonisation of the mouse respiratory tract. Bacterial uptake into alveolar macrophages relies on selectin and integrin mimicry by PT and FHA, respectively. Pertussis toxin S2 and S3 treatment of human macrophages upregulates CR3 as a result of selectin mimicry (selectins have been shown to upregulate integrins). Secreted FHA, which has binding sites for both integrins and the bacterial surface itself, coats the bacteria and mediates CR3 binding and phagocytosis, permitting avoidance of the respiratory burst and intracellular multiplication. Pertussis toxin has also been shown to bind the Mac-1 integrin receptor (CD11b/CD18) leading to tyrosine phosphorylation and monocytic cell adhesion in serum.

Several proteins secreted by a type IV pathway (autotransporters) contain RGD motifs, which are associated with attachment to mammalian cells via binding of β integrins on the plasma membrane. These include B. pertussis brkA, P.69 pertactin, and tcfA, Bordetella parapertussis P.70, Bordetella bronchiiseptica P.68, E coli antigen 43, and Serratia marcescens ssp. Most RGD containing autotransporters contain two RGD sites, one within the passenger domain and one within the β domain. The importance of these RGD motifs has yet to be determined, but they probably play a role in the adhesion of B. pertussis P.69 pertactin.

Bartonella bacilliformis is a fastidious Gram negative bacillus, transmitted by the sandfly, which causes bartonellosis. It is a facultative
Table 3 Viruses that interact with eukaryotic cell adhesion molecules (CAMs) during their pathogenesis

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<th>Eukaryotic CAM</th>
<th>Role in pathogenesis</th>
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<td>Poliovirus 1–3</td>
<td>PVR (Ig-like)</td>
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<td>Coxsackie virus A13, A18, A21</td>
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<tr>
<td>Coxsackie virus A9</td>
<td>α,β integrins</td>
<td>Adhesion and invasion</td>
<td>88</td>
</tr>
<tr>
<td>Coxsackie virus B1–6</td>
<td>CAR (Ig-like)</td>
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<td>Coxsackie virus B1, B3, B5</td>
<td>DAF, α,β integrins</td>
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<td>Adenovirus</td>
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<td>Echovirus 1</td>
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<td>Echovirus 3, 6, 7, 11–13, 20, 21, 24, 29, 33</td>
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<td>Echovirus 22</td>
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<td>Hepatitis A (enterovirus 72; HAV)</td>
<td>HAVcr-1 (Ig-like)</td>
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<td>Rhinovirus major gp (91 serotypes)</td>
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<td>Foot and mouth disease virus</td>
<td>α,β Integrin</td>
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<td>Adhesion and invasion of T lymphocytes; downregulation of CD4</td>
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<td>Immune evasion by downregulation of α,β integrin on fibroblasts</td>
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<tr>
<td>Adeno associated virus (AAV)</td>
<td>α,β Integrin</td>
<td>Adhesion and invasion</td>
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<tr>
<td>Hantaviruses</td>
<td>α,β Integrins</td>
<td>Adhesion and invasion of platelets and endothelial cells</td>
<td>90</td>
</tr>
<tr>
<td>Bovine leukaemia virus</td>
<td>CD11c integrin</td>
<td>Pathogenesis of lymphocytosis involves upregulation of CD11c on B and T lymphocytes</td>
<td>111</td>
</tr>
</tbody>
</table>

CAR, coxsackie adenovirus receptor; DAF, decay accelerating factor; HIV, human immunodeficiency virus; ICAM-1, intercellular adhesion molecule 1; PMN, polymorphonuclear leucocyte; PVR, poliovirus receptor; VCAM-1, vascular cell adhesion molecule 1.

Intracellular pathogen that binds to α,β integrin on human epithelial cells, with the induction of lysosome phosphorylation of several host proteins and subsequent uptake.69

*Pasteurella haemolytica* is a Gram negative bacillus that produces a leucotoxin that kills bovine immune cells and causes bovine pasteurellosis. The leucotoxin is a member of the RTX family of pore forming toxins, which are thought to effect cellular killing by induction of apoptosis. An early step for induction of apoptosis is the binding of leucotoxin to β1 integrin on bovine leucocytes.70

The Lyme disease spirochaete, *Borrelia burgdorferi*, is transmitted by the bite of an infected tick, causing localised infection, which disseminates, leading to chronicity. It is a facultative intracellular pathogen, and binds CR3 leading to CR3 phagocytosis.71 The organism also binds platelets via α,β integrin, which may be important in dissemination.72

*Porphyromonas gingivalis* is an anaerobic Gram negative bacillus crucial for the development of adult periodontitis. *Porphyromonas gingivalis* lipopolysaccharide (LPS) has been shown to upregulate E selectins and P selectins, among other activities,73 and this might be important in colonisation and pathogenicity. *Actinobacillus actinomycetemcomitans*, another coloniser of the gingival sulcus and periodontal pathogen, enters host cells through ruffled apertures by an actin dependent mechanism, which may depend on host cell integrins for internalisation.74 *Actinobacillus actinomycetemcomitans* also produces a leucotoxin that is a member of the RTX family of toxins. The leucotoxin binds LFA-1, a β1 integrin, on leucocytes, resulting in pore formation and cytolysis.75

Mycoplasmas are minute prokaryotes without a cell wall, which enter an appropriate host and multiply and survive for extended periods of time. Mycoplasmas stimulate lymphocytes in a non-specific polyclonal manner. For example, *Mycoplasma penetrans* possesses several B and T cell mitogens, including a membrane lipoprotein whose activity is inhibited in a dose dependent manner by a monoclonal antibody against CD18, the β2 subunit of β integrins.76 In addition, *Mycoplasma arthritidis* produces a superantigen.77

**VIRUSES**

Table 3 summarises interactions of eukaryotic CAMs with viruses. Replication of a virus begins with attachment to its receptor (a eukaryotic cell surface molecule), followed by entry of the virus into the cell, and there are many examples of the use of CAMs in viral pathogenesis. Picornaviruses utilise at least nine distinct receptors for cell entry.78 All three serotypes of poliovirus bind to the poliovirus receptor (PVR), which is a three immunoglobulin-like domain type I membrane glycoprotein, whose cellular function is unknown.79 At present, no other picornavirus is known to use this receptor.80 Coxsackie virus A9 (CVA9) uses the vitronectin receptor (α,β integrin), also used by hantaviruses81 and foot and mouth disease virus.82 CVA9 has an RGD binding motif at the carboxyl end of VP1 (viral protein 1), and virus binding is blocked by RGD containing peptides; however, because trypsin mediated inactivation of this motif does not block infection of African green monkey cells, there may be an alternative receptor.83 ICAM-1 is used by coxsackie viruses A13, A18, and A2184 and the major group of rhinoviruses (91 serotypes).85 86 The coxsackie viruses, especially CBV3, may use decay accelerating factor (DAF) in association with α,β integrin,87 88 or the immunoglobulin-like coxsackievirus adenovirus receptor (CAR), also used by the adeno viruses.89 Echoviruses use α,β integrin,89 DAF88–100 or α,β integrin.101 102 Hepatitis A (enterovirus 72; HAV) uses the HAV cellular receptor 1 (HAVcr-1), whose cellular function is unknown, although is known to be immunoglobulin and mucin-like.103 Encephalomyocarditis virus uses VCAM-1 for adhesion and invasion.104

Human immunodeficiency virus 1 (HIV-1) gp120 binds CD4, a member of the immunoglobulin superfamily, on mature circulating T helper/inducer lymphocytes as a first step in...
molecule; PSGL, P selectin glycoprotein ligand; PMN, polymorphonuclear leucocyte; TNF-α, tumour necrosis factor α; IFN-γ, interferon γ; ESL, E selectin ligand; GlyCAM-1, glycosylation dependent cell adhesion molecule 1; IAP, integrin associated protein; ICAM-1, intercellular adhesion molecule 1.

Table 4 Human cell adhesion molecules (CAMs) mediating leucocyte–endothelial cell interaction during host defence against infection

<table>
<thead>
<tr>
<th>Leucocyte receptor</th>
<th>Leucocyte distribution</th>
<th>Endothelial ligand</th>
<th>Expression</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selectins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L selectin (CD62L)</td>
<td>All leucocytes</td>
<td>GlyCAM-1 and others</td>
<td>Constitutive</td>
<td>Rolling</td>
</tr>
<tr>
<td>aβ (Mac-1, Mo-1, CD11b/CD18)</td>
<td>Monocytes, PMN</td>
<td>ICAM-1, ICAM-2</td>
<td>Induced by IFN-γ and others</td>
<td>Firm adhesion</td>
</tr>
<tr>
<td>aβ (LFA-1, CD11a/CD18)</td>
<td>All leucocytes</td>
<td>ICAM-1</td>
<td>Induced by IFN-γ</td>
<td>Firm adhesion</td>
</tr>
<tr>
<td>αβ (CD49d/CD29)</td>
<td>All leucocytes</td>
<td>VCAM-1</td>
<td>Constitutive</td>
<td>Rolling</td>
</tr>
<tr>
<td>αβ (Lympocytes)</td>
<td>MacCAM</td>
<td></td>
<td>Induced by TNF-α and others</td>
<td>Firm adhesion or rolling</td>
</tr>
<tr>
<td>αβ (PMN)</td>
<td>Lympocytes, PMN</td>
<td>PECAM (CD31)</td>
<td>Constitutive</td>
<td>Transmigration</td>
</tr>
<tr>
<td>Immunoglobulin superfamily</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IAP (CD47)</td>
<td>All leucocytes</td>
<td>?</td>
<td>Constitutive</td>
<td>Transmigration</td>
</tr>
<tr>
<td>PECAM (CD31)</td>
<td>PMN</td>
<td>PECAM ? αβ</td>
<td>Constitutive</td>
<td>Transmigration</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESL</td>
<td>PMN</td>
<td>P selectin (CD62P)</td>
<td>Constitutive</td>
<td>Rolling</td>
</tr>
<tr>
<td>CD44</td>
<td>All leucocytes</td>
<td>Hyaluronate</td>
<td>Induced by IFN-γ</td>
<td>Function</td>
</tr>
</tbody>
</table>

ESL, E selectin ligand; GlyCAM-1, glycosylation dependent cell adhesion molecule 1; IAP, integrin associated protein; ICAM-1, intercellular adhesion molecule 1; IFN-γ, interferon γ; ESL, E selectin ligand function associated antigen 1; MacCAM, mucosal address cell adhesion molecule; PECAM, platelet endothelial adhesion molecule; PSGL, P selectin glycoprotein ligand; PMN, polymorphonuclear leucocyte; TNF-α, tumour necrosis factor α; VCAM, vascular cell adhesion molecule 1.

infection. In addition, CD4–gp120 interaction is necessary for cell–cell spread of HIV. This led to early investigation of recombinant soluble CD4 (rsCD4) as an anti-HIV treatment. High dose intravenous rsCD4 abrogates viraemia in a dose dependent manner; however, the duration of the effect is limited by the pharmacokinetics of rsCD4. Although pharmacokinetics have been improved by combining rsCD4 with immunoglobulin molecules (rsCD4–IgG), therapeutic benefit in the treatment of established HIV infection has been limited. However, there may be some benefit in prophylaxis of perinatal and needle stick infections. In addition, dysregulated neutrophil function in HIV infection is mediated by integrins and selectins.

Human herpesvirus 7 (HHV7) grows well in CD4 positive lymphocytes and also uses the CD4 molecule as its receptor. HHV7 also downregulates expression of CD4 and, therefore, the use of its envelope has been suggested in anti-HIV treatment.

Cytomegalovirus (CMV), another herpesvirus, achieves permanent coexistence with its hosts by establishing latent infection in specific “privileged” tissues to evade immune surveillance. Mechanisms to this end include the CMV mediated downregulation of αβ integrin in human fibroblasts, and the possible production of a putative superantigen that is present on infected monocytes, leading to selective expansion of Vβ T cell receptor bearing lymphocytes. Alternatively, this superantigen may be a human gene product transcriptionally activated by CMV expression, which may induce active infection from the latent state.

Adeno associated virus 2 (AAV-2), a ubiquitous human parovirus, which is used as a vector in various experimental gene therapies, uses HSPG as a primary receptor, but is also dependent on αβ integrin to increase susceptibility of cells to infection. αβ Integrin is involved in facilitating virus internalisation, a finding that has relevance for human gene therapy.

Bovine leukaemia virus (BLV), human T lymphotropic virus 1 (HTLV-1), and HTLV-II belong to the same family of oncogenic retroviruses that causes spontaneous lymphocyte proliferation. Infection with these viruses is associated with increased expression of lymphocyte integrins, CD11c and CD18. Lymphocyte dysregulation in spontaneous lymphocyte proliferation probably involves signalling through these lymphocyte surface molecules.

CAMs in the host defence against infection

The cellular immune system is a potent force which, if controlled and directed correctly, is effective against an enemy and forms an appropriate host defence; however, if uncontrolled, it is ineffective against the enemy and can actually be harmful to the host itself. Therefore, leukocyte action must be held in check in the circulation, and discharged at the site of infection. This is achieved through the leukocyte adhesion molecules, which recognise areas of tissue damage and direct their own transendothelial migration into the tissues. Important in this process are integrins, selectins, and immunoglobulin-like superfamily members.

Leucocyte adhesion molecules

Leucocyte migration to sites of infection requires several separate adhesion events, namely: tissue damage recognition, endothelial interaction, transendothelial migration, and migration through the extracellular matrix to the site of infection. CAM families that mediate leucocyte and endothelial cell interaction and which are primarily responsible for these events include the integrins, selectins, and immunoglobulin superfamily. They recognise each other as a result of their affinities and their location on the plasma membrane. Table 4 summarises their expression and interactions. Because of the constitutive expression of certain leucocyte CAMs and the expression of certain other CAMs by the endothelial cells only at sites of inflammation, the leucocyte is always ready to recognise the endothelium at sites of infection.

Integrins exist in the membrane as heterodimers and function in cell–cell and cell–matrix interactions in almost all cells. Each heterodimer consists of an α and β chain encoded by their respective genes. There are 14 known α genes and eight known β genes, which
can combine to express 22 different integrins. The integrins modulate ligand binding kinetics in response to environmental signals and facilitate the need of leucocytes for migration, flexibility of function, and rapid response. Several leucocyte integrins are crucial to leucocyte adhesion, rolling, and transmigration, such as α<sub>4</sub>β<sub>1</sub>, α<sub>5</sub>β<sub>1</sub>, α<sub>3</sub>β<sub>1</sub>, α<sub>5</sub>β<sub>2</sub>, and α<sub>β</sub>. (Table 4).

Selectins are calcium dependent, and expressed on leucocytes and endothelial cells. L selectins are expressed constitutively on virtually all leucocytes and E selectins and P selectins are expressed on endothelial cells. E selectin is not expressed on resting endothelium but is expressed after stimulation by a number of cytokines released in response to inflammation, tissue damage, and immune responses. P selectin is expressed constitutively in many endothelial cells; not at the plasma membrane, but in regulated secretory Wiebel-Palade bodies. This stored P selectin is rapidly mobilised to the plasma membrane in response to inflammatory stimuli, such as thrombin or histamine, and is then recognised by circulating leucocytes. All known specific ligands for the selectins are mucins.

Immunoglobulin superfamily members mediate cell adhesion for a variety of different cell types. For example, on endothelial cells, ICAM-1 and ICAM-2 are recognised by leucocyte integrins; VCAM-1 is a ligand for α<sub>β</sub>, integrin on monocytes and lymphocytes, and mucosal addressin CAM (MAdCAM) is recognised by α<sub>β</sub>, and L-selectin on Peyer’s patch lymphocytes, and has both integrin binding immunoglobulin domains and a selectin binding mucin domain. Two immunoglobulin superfamily members involved in leucocyte transendothelial migration are platelet endothelial adhesion molecule (PECAM) and integrin associated protein (IAP). PECAM is expressed at endothelial cell–cell junctions and on all leucocytes. IAP is present on fibroblasts, platelets, erythrocytes, neurones, epithelium, endothelium, and leucocytes. Both PECAM and IAP are expressed on leucocytes and endothelium and are probably involved in leucocyte transendothelial migration.

**Leucocyte transendothelial migration**

The current leucocyte–endothelial adhesion model involves three steps. In the first step, leucocytes are slowed in the circulation, most often by low affinity selectin–mucin interaction. This leads to leucocyte rolling along the vessel wall, with increased exposure of the leucocyte to the vessel environment, and thus increased sampling of signals from cytokines, chemotactants, lipopolysaccharide, or abnormal homeostasis. Prevention of the rolling process severely impedes migration. Rolling leads to leucocyte activation as a result of stimulation of receptors, which include the serpentine family. Leucocyte adhesion deficiency (LAD) type II comprises a syndrome of mental retardation, short stature, and defective polymorphonuclear cell function caused by an absence of sialyl-Lewis-X, an essential part of selectin ligands, which results in greatly decreased leucocyte rolling.

The second step is endothelial adhesion mediated by high avidity integrin activation, which for its efficient recruitment requires prior leucocyte rolling. This adhesion is inhibited by anti-β<sub>2</sub> integrin antibodies and is defective in LAD type I, a syndrome of delayed umbilical cord separation, and recurrent bacterial infections caused by a defect in polymorphonuclear cell migration and activation. LAD type I is caused by a defect in the β<sub>2</sub> chain of the leucocyte β<sub>2</sub> integrin. It has been suggested that PECAM on tightly adherent leucocytes binds endothelial α<sub>5</sub>β<sub>2</sub> with a consequent loosening of cell–cell junctions, allowing the leucocytes to migrate through. Once they have left the vascular compartment, leucocytes migrate to sites of infection on extracellular matrix proteins.

**Leucocyte activation at sites of infection**

Leucocytes at sites of infection and inflammation have a very different phenotype from those present in the circulation: they are metabolically active, highly adherent, and destructive. Many mediators can signal this phenotypic change, but among the most potent are the proteins of the ECM. This mechanism depends on β<sub>2</sub> integrin as the leucocyte receptor; called the leucocyte response integrin. IAP probably forms a complex with the leucocyte response integrin on the plasma membrane, a complex that acts as the signalling unit through which the ECM proteins mediate polymorphonuclear cell activation. Integrins also cooperate with other host defence receptors, such as F<sub>c</sub>R and Mac-1.

**Conclusion and future prospects**

In conclusion, eukaryotic CAMs are essential for the host defence against infection, and from present knowledge they are an integral facilitating factor in the pathogenesis of many protozoal, fungal, bacterial, and viral infections. Currently, the number of antibiotic options for treatment of infectious disease are limited because of the development of resistance. This has led to measures to preserve the usefulness of antibiotics, develop new classes of antibiotic, and develop alternative strategies for the treatment of infectious disease—for example, human recombinant antibodies, bacteriophage treatment, and the identification of novel bacterial targets for antibiotic treatment and for passive and active immunisation. The CAMs may represent such a target. For example, the RGD motif occurs in many microbial proteins and mediates binding to human integrins. RGD peptides and mimetics not only provide insights into microbial pathogenesis, but are potential therapeutics for various infections.
tious diseases, in addition to thrombosis and cancer.13

The complete genome sequence is known for 10 bacterial pathogens and ~30 more are anticipated by the year 2000 (http://www.tigr.org). Comparative genomics, increasingly important, has provided the knowledge that shared virulence determinants exist in several different bacterial genera, and that the pathogens among these have modified these determinants to perform particular functions and therefore facilitate their particular lifestyles. Currently, using nucleotide sequence data, we can compare entire microbial genomes and identify putative virulence factors with homologous function. In addition, the human genome mapping project will provide insights into the pathogenesis of microbial infection, particularly in the context of the interaction of prokaryotic and eukaryotic molecules. With improvements in animal modeling and probes for global microbial gene expression under different conditions such as microarrays, our understanding of the pathogenesis of infectious disease and its treatment and prevention will continue to improve.13

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