Microbial utilization of human signalling molecules

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Overview

Many pathogenic micro-organisms specifically target host-cell receptors that normally mediate intercellular interactions, attachment to substrata, or receive hormonal, cytokine and other environmental signals. A fundamental question in microbial pathogenesis is how such targeting leads to cellular invasion. In eukaryotic cells, signal transduction involves a complex array of interactive molecules and second messengers that control multiple cellular functions. In this review, several eukaryotic signal transduction systems currently known are presented briefly to set the scene for discussing examples of distinct receptor/signalling systems that are known to be manipulated by pathogenic microbes. The second part of the review will focus on one large subset of receptors, the adhesion molecules, and explore the mechanisms by which microbes engage with them during the course of pathogenesis.

Signalling pathways of eukaryotic cells and some examples of their subversion by pathogens

In broad terms, two distinct mechanisms of eukaryotic transmembrane signal transduction are recognized: (a) unimolecular receptor/effector systems that span the plasma membrane with the ligand-binding domain exposed at the cell surface and enzymic effector domain at the cytoplasmic face, and (b) multi-component systems with independent but coupled receptors, transducers and effector molecules. In each case, signals originating at the membrane are passed on to cytosolic molecules in a cascade of activating events and eventually to the nucleus. At the heart of these pathways are phosphorylating enzymes whose compartmentalization is critical in specific signal transduction and in part depends on kinase-anchoring proteins located at various sites in the cell (Mochly-Rosen, 1995). Although distinct pathways may be activated via individual ligand–receptor interactions, many are interrelated (Fig. 1). Moreover, multiple receptor systems can interact and synergize with each other to regulate cellular events (Rosales et al., 1995; Clark & Brugge, 1995).

Main categories of eukaryotic signalling pathways

Receptor tyrosine kinase pathway (RTK)/Ras pathway

RTK pathways have tyrosine kinase activity directly associated with the receptor as a part of the receptor molecule. The membrane-located proto-oncogene product Ras is central to this pathway. Ras is a low-molecular-mass GTP-binding protein (small G-protein) whose activation involves conversion from GDP to GTP-bound (activated) form. Binding of ligand (usually growth factors) results in receptor clustering, activation of cytoplasmically located tyrosine kinase domain and autophosphorylation of tyrosine residues at the C terminus. This leads to binding of other proteins such as Grb-2/Sos complex (cytosolic complexes with affinity for phosphorylated receptor as well as Ras) to the receptor and to plasma-membrane-located Ras. Sos activates Ras via exchange of GDP for GTP, leading to activation of other proteins downstream in the cascade which include cytoplasmic serine/threonine kinases (Raf-1, MEK and MAP-kinases). The latter can migrate from the cytosol to the nucleus and deliver signals to the nucleus by phosphorylation of transcription factors. Direct activation of the nuclear factor NF-kB may occur via Raf-1 which can phosphorylate IκB, the inhibitor of NF-kB. Negative modulation of Ras occurs via GAPs, GTPase-activating proteins, that stimulate intrinsic GTPase activity of Ras (Maruta & Burgess, 1994). Recent evidence suggests that the cAMP and other second messenger signalling pathways are interconnected with the RTK pathway (Rosales et al., 1995). It appears that phosphorylation of tyrosine plays a key role in Ras activation since other tyrosine kinase pathways (see below) also appear to activate Ras (Maruta & Burgess, 1994).

The superfamily of small GTPases include five classes of proteins: Ras (primarily affects cell growth and development), Ran (involved in nuclear protein import), Rab and Arf (monitor and direct vesicular movement) and the
Rho subfamily (involved in regulation of the actin cytoskeleton). Within the Rho subfamily, different proteins participate to direct distinct patterns of actin reorganization. Rho proteins are associated with focal adhesions, Rac with membrane ruffling and NADPH oxidase regulation in some cells, and Cdc42 with actin-containing microspikes or filopodia formation (Aktories & Just, 1995; Vojtek & Cooper, 1995).

Non-receptor tyrosine kinase (NRTK) pathways

These pathways are used by receptors often involved in immune recognition. Activation requires receptor aggregation and non-covalent association with other membrane-located proteins such as Src. Src kinases are acylated membrane-associated proteins with Src homology (SH) domains characteristic of many molecules within signalling pathways and which mediate physical association with other components. For example, SH2 domains recognize motifs containing phosphotyrosine, whilst SH3 domains bind to short proline-rich regions (Seed, 1995). Src-like kinases have also been implicated in integrin signalling events (Clark & Brugge, 1995) and in activation of RTK as well as receptors coupled to G-proteins (Erpel & Courtneidge, 1995). Other NRTKs are Syk kinases of haematopoietic cells which are activated by β1 and β3 integrin cross-linking (Clark & Brugge, 1995). Full activation of Src family kinases requires two opposing enzyme activities: the removal of C-terminal phosphate and phosphorylation of tyrosine proximal to the active site (Seed, 1995).

G-protein pathways

These are utilized by many classes of hormones and involve heptahelical receptors (with seven hydrophobic transmembrane regions, also known as serpentine receptors, Serp-R) (Fig.1) that couple to heterotrimeric G-proteins which are distinct from small monomeric G-proteins. These membrane-associated trimers consist of α, β, and γ subunits. The Gβ and Gγ subunits control the function of the Ga subunit (Nurnberg et al., 1995). The Ga subunit binds GTP, has GTPase activity and is the effector molecule. Its activation results in activation of other effector enzymes to generate second messengers such as cAMP, 1,2 diacylglycerol (DAG) and inositol triphosphate (IP₃) or to regulate ion channels (Nurnberg et al., 1995). These second messengers are involved in activating protein kinases directly or via Ca²⁺/calmodulin complexes, leading to activation of cellular events. Specificity and selectivity of signalling via G-proteins is accomplished by coupling of receptors with distinct G-protein trimers composed of variants of different subunits. Some receptors can activate multiple G-proteins, thus
regulating diverse signal-transduction pathways (Roses et al., 1995; Nürnberg et al., 1995).

Pathways used by adhesion molecules

Cytoskeleton-linked pathways (integrin-associated signalling). Integrins are a superfamilly of heterodimeric trans-membrane molecules consisting of one of 16 α chains and one of eight β chains giving rise to more than 20 different receptors (Clark & Brugge, 1995). Integrin families and their normal ligands are described in Table 1. Integrins have short cytoplasmic tails without catalytic domains that associate with cytoskeletal proteins such as α-actinin, talin, vinculin, paxillin and tensin in complexes (focal adhesions, Fig. 2) and affect cytoskeletal arrangement via actin microfilaments (Clark & Brugge, 1995). The extracellular domains have Ca²⁺-binding sites (Fig. 2) implicated to participate in ligand binding. Several integrins interact with matrix proteins and thus have the capacity of forming a physical link between the extracellular matrix (ECM) and cytoskeleton. Two principal mechanisms by which integrins are activated to transduce signals involve conformational change and receptor clustering. Conformation-dependent activation is exemplified by thrombin-mediated activation of gpIIb/IIIa integrin expressed on platelets. Thrombin induces intracellular signalling leading to modulation of the conformation of gpIIb/IIIa, which is then able to bind fibrinogen leading to platelet aggregation. This has been called `inside out' signalling (Richardson & Parsons, 1995).

Signal transduction via integrins may reside, at least in part, in cytoskeletal rearrangement which may physically bring together molecular complexes by forming focal adhesions. Inhibition of clustering, which is achieved via cytoskeletal rearrangement, also inhibits tyrosine kinases suggesting kinase activation may be mediated by receptor clustering (Clark & Brugge, 1995). A tyrosine kinase, focal adhesion kinase (FAK) pp125FAK, is believed to play an important role in integrin-mediated signal transduction (Roses et al., 1995; Clark & Brugge, 1995). This kinase localizes to focal adhesion complexes and is stimulated to autophosphorylate at Tyr³⁹⁷ upon integrin binding to ECM. Studies using the actin-depolymerizing agent cytochalasin D have shown that signal transduction via integrins involves MAP kinase activation in addition to pp125FAK phosphorylation and also that signal transduction via integrins requires cytoskeletal reorganization (Roses et al., 1995). FAK may interact with Src and recruit Src to focal adhesions causing hyper-phosphorylation of focal adhesion structures. There is also evidence for association of other tyrosine kinases with integrins, for example, in monocytes, Syk (Srcfamily) may respond to integrin signalling. Integrin binding to ECM also leads to a rise in intracellular Ca²⁺ via a 50 kDa integrin-associated protein, IAP shown to bind to some integrins (Roses et al., 1995).

Stimulation of integrins may be translated into a variety of intracellular signals. For example, monocyte adherence to fibronectin or collagen activates transcription of inflammation mediators such as IL-1β and IL-8. Integrins may also act in concert with other receptor pathways to enhance or dampen signals. In particular, activation via growth factor receptors require adherence of cells to ECM via integrins. The small G-protein Rho appears to be critical in integrating signals induced by integrins and growth factor receptors (Clark & Brugge, 1995). Protein tyrosine phosphatases (PTPases) also appear to be involved in regulation of cell adhesion which may involve dephosphorylation of pp125FAK, implicating further the role of Rho in integrin-mediated adhesion (Roses et al., 1995). In addition, PTPases may activate Src kinases by dephosphorylating the negative-regulatory C-terminus phosphotyrosine (Clark & Brugge, 1995).

The complexities of integrin/cytoskeletal signalling pathways are discussed in comprehensive reviews by Roses et al. (1995) and Clark & Brugge (1995).

Pathways used by other adhesion molecules. Mechanisms and transduction pathways used by the immunoglobulin (Ig) superfamilly, cadherins and selectins are much less well understood at present compared with integrin pathways. Structural studies on certain families of transmembrane tyrosine kinases such as the fibroblast growth factor receptor (FGFR) and tyrosine phosphatases have revealed Ig motifs in their extracellular domains. These observations may allude to signal transduction mechanisms of Ig superfamily receptors. Studies on NCAM and N-cadherins suggest that these can act via tyrosine kinases, G-proteins and arachidonate metabolites. The latter may act as second messengers and activate calcium channels. Cadherins also bind to intracellular proteins called catenins. Tyrosine phosphorylation of catenins or other molecules leads to the detachment of cell-cell contacts mediated by cadherins and results in disruption of epithelial integrity. Additionally, evidence has been provided that adhesion receptors may interact directly with the RTKs such as FGFR (Friesel & Maciag, 1995; Roses et al., 1995).

The sphingolipid signalling pathway

The agonists that activate this pathway include TNF-α, interferon, IL-1 and vitamin D3. Ligand engaging with the receptor results in activation of a sphingomyelinase that cleaves sphingomyelin to release ceramide and phosphocholine. Ceramide stimulates protein kinases and protein phosphatases leading to activation of transcription factors such as c-jun and NF-xB. It has been implicated in negative regulation of cell growth and in apoptosis (Roses et al., 1995; Divecha & Irvine, 1995).

Other pathways

Signal transduction via cytokines involves a family of cytoplasmic tyrosine kinases (Janus kinases, JAKs). Two JAKs are required to activate each other by reciprocal transphosphorylation (Schindler, 1995). These can activate Ras signal pathway (Roses et al., 1995) or directly phosphorylate tyrosine in cytoplasmic proteins [termed STATs (signal transducers and activators of transcription)] which migrate to the nucleus and affect transcription of specific genes (Roses et al., 1995; Schindler, 1995).
### Table 1. Some known adhesion molecule receptors of pathogenic microbes

<table>
<thead>
<tr>
<th>Family/member</th>
<th>Natural ligand*</th>
<th>Microbe/ligand</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Integrin superfamily</strong>&lt;br&gt;β1 Integrins [very late activation antigen, VLA (CD29)]&lt;br&gt;VLA-2: α2β1</td>
<td>Col, Lm</td>
<td>Echovirus 1</td>
<td>Bergelson et al. (1992)</td>
</tr>
<tr>
<td>VLA-3: α3β1</td>
<td>Fn, Lm, Col</td>
<td>Yersinia invasin</td>
<td>Isberg &amp; Tran Van Nhieu (1994)</td>
</tr>
<tr>
<td>VLA-4: α4β1</td>
<td>Fn, VCAM-1, LFA-1, CR3</td>
<td>Yersinia invasin</td>
<td>Isberg &amp; Tran Van Nhieu (1994)</td>
</tr>
<tr>
<td>VLA-5: α5β1</td>
<td>Fn</td>
<td>Yersinia invasin</td>
<td>Isberg &amp; Tran Van Nhieu (1994)</td>
</tr>
<tr>
<td>VLA-6: α6β1</td>
<td>Lm</td>
<td>HIV-1 Tat protein &lt;br&gt;(Neisseria meningitidis Opc protein)</td>
<td>Barillari et al. (1993)</td>
</tr>
<tr>
<td>αvβ1</td>
<td>Fn</td>
<td>Shigella flexneri Ipa proteins</td>
<td>Watarai et al. (1996)</td>
</tr>
<tr>
<td><strong>β2 Integrins (leucams; LFA-1, CD18 group)</strong>&lt;br&gt;LFA-1: αLβ2</td>
<td>ICAM-1/2/3</td>
<td>Histoplasma capsulatum</td>
<td>Wright et al. (1989)</td>
</tr>
<tr>
<td>(CD11a/CD18)</td>
<td></td>
<td>Leishmania gp63, LPG, LPS</td>
<td>Russell &amp; Wright (1988)</td>
</tr>
<tr>
<td>CR3, Mac-1: αmβ2</td>
<td>ICAM-1, C3bi, Fbg, Factor X</td>
<td>Histoplasma capsulatum, Legionella pneumophila, Bordetella pertussis FHA, Rhodococcus equi, Escherichia coli type I fimbriae</td>
<td>Wright et al. (1989)</td>
</tr>
<tr>
<td>(CD11b/CD18, Mo-1)</td>
<td></td>
<td>Histoplasma capsulatum, LPS</td>
<td>Gbarah et al. (1991)</td>
</tr>
<tr>
<td>CR4, P150,95: αβ2</td>
<td>ICAM-1, Fbg</td>
<td>Leishmania LPG</td>
<td>Talamas-Rohana et al. (1990)</td>
</tr>
<tr>
<td>(CD11c/CD18, Leu M5)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>β3 Integrins (cytoadhesins)</strong>&lt;br&gt;Platelet glycoprotein IIbIIIa: αIIbβ3&lt;br&gt;Vitronectin receptor (VNR): αvβ3 (CD51)</td>
<td>Fbg, Fn, vWF, Vn, Tsp</td>
<td>Borrelia burgdorferi</td>
<td>Coburn et al. (1993)</td>
</tr>
<tr>
<td></td>
<td>Vn, vWF, Fbg, Tsp</td>
<td>Adenovirus penton base</td>
<td>Wickham et al. (1993)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIV-1 Tat protein</td>
<td>Barillari et al. (1993)</td>
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<td></td>
<td></td>
<td>Coxackievirus A9</td>
<td>Roivainen et al. (1994)</td>
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<td></td>
<td></td>
<td>Mycobacteria avium-intracellulare</td>
<td>Rao et al. (1993)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neisseria meningitidis Opc protein</td>
<td>Virji et al. (1994a)</td>
</tr>
<tr>
<td><strong>β5 Integrins</strong>&lt;br&gt;αvβ5</td>
<td>Vn</td>
<td>Adenovirus penton base</td>
<td>Wickham et al. (1993)</td>
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</tbody>
</table>
**Table 1. (cont.)**

<table>
<thead>
<tr>
<th>Family/members</th>
<th>Natural ligand*</th>
<th>Microbe/ligand</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Immunoglobulin superfamily</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVR</td>
<td>?</td>
<td>Poliovirus</td>
<td>Mendelsohn et al. (1989)</td>
</tr>
<tr>
<td>CD4</td>
<td>Major histocompatibility complex (MHC) Class II</td>
<td>HIV-1,2; SIV envelope glycoprotein gp120</td>
<td>Harrison (1994)</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>LFA-1, Mac-1</td>
<td>Human Rhinovirus (HRV)</td>
<td>Greve et al. (1989)</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>VLA-4, VLA-4</td>
<td>Coxsackievirus A (CAV)</td>
<td>Crowell &amp; Tomko (1994)</td>
</tr>
<tr>
<td>NCAM (CD56)</td>
<td>Heparan sulphate NCAM</td>
<td>Plasmodium falciparum</td>
<td>Pasloske &amp; Howard (1994)</td>
</tr>
<tr>
<td>CEA (CD66)</td>
<td>CD66, selectins</td>
<td>Neisseria meningitidis and Escherichia coli K1 capsules — molecular mimicry</td>
<td>Finne et al. (1983, 1987)</td>
</tr>
<tr>
<td>Selectin family (LECCAM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cadherins</td>
<td>LAM</td>
<td>Shigella flexneri</td>
<td>Sansonetti et al. (1994)</td>
</tr>
<tr>
<td></td>
<td>LAM</td>
<td>Listeria monocytogenes</td>
<td>Mengaud et al. (1996)</td>
</tr>
<tr>
<td>Other</td>
<td>CD36</td>
<td>Plasmodium falciparum</td>
<td>Pasloske &amp; Howard (1994)</td>
</tr>
<tr>
<td></td>
<td>CD44 (HCAM, Hermes antigen)</td>
<td>Poliovirus</td>
<td>Shepley &amp; Racaniello (1994)</td>
</tr>
</tbody>
</table>

* Abbreviations: CEA, carcinoembryonic antigen; Col, collagen; Lm, laminin; Fn, fibronectin; Fbg, fibrinogen; Vn, vitronectin; vWF, von Willebrand factor; Tsp, thrombospondin.

**Signalling pathways manipulated by micro-organisms**

Numerous studies have investigated signalling systems utilized by different micro-organisms. Rather than provide an exhaustive list, a small number of recent studies primarily on bacteria are chosen to illustrate a range of signalling pathways used and resulting distinctive outcomes of microbial signalling.

**Distinct modes of cellular invasion via induced cytoskeletal events**

Many enteropathogenic bacteria (some strains of *Escherichia coli*, *Salmonella*, *Shigella*, *Yersinia*, *Listeria*) as well as other mucosal pathogens (*Haemophilus influenzae* type b and the pathogenic *Neisseriae*: *N. meningitidis* and *N. gonorrhoeae*) induce endocytosis in host epithelial or endothelial cells (parasite-directed phagocytosis). Signal transduction events in many cases, although utilizing different receptors, are linked to cytoskeletal rearrangement, often involve tyrosine phosphorylation of host proteins and changes in intracellular second messengers. Actin polymerization is a frequent requirement since cytochalasins inhibit invasion. Several enteric pathogens (*Salmonella*, *Shigella*) induce pronounced cytoskeletal rearrangements and induce invasion via cellular 'triggering'. Others (*Yersinia*) induce more localized actin polymerization and this more quiescent invasion is described as 'zippering'. A third class of pathogens, exemplified by *Trypanosoma cruzi*, do not require actin polymerization and their invasion may be enhanced in the presence of cytochalasin D (Rosenshine & Finlay, 1993; Bliska et al., 1993; Rodriguez et al., 1995).

*Salmonella* and *Shigella* share some similarities in their mechanisms of inducing extensive ruffling of their target cell membrane. Both secrete multiple protein complexes to trigger their uptake (Menard et al., 1996). Several of the induced signal transduction events in the host cell are, however, distinct. In the case of *Salmonella*, a marked increase in intracellular calcium occurs and is necessary for invasion (Bliska et al., 1993). Cellular entry in some cells may involve EGF receptor and tyrosine...
phosphorylation (Bliska & Falkow, 1993). More than one signal transduction pathway of the host may be used via yet largely undefined Salmonella ligand–host-receptor interactions (Bliska et al., 1993).

Shigella-induced plasma membrane activity is triggered by Ipa antigens. Involvement of the integrin/cytoskeleton pathway including pp125<sup>FAK</sup>, has been implicated recently in Shigella flexneri interactions with mammalian cells (Watarai et al., 1996). Endocytosis was mediated by released bacterial proteins (IpaB, IpaC and IpaD) which bound to integrin α5β1 in a manner similar to the tissue form of fibronectin. At the site of attachment of bacteria, the integrin α5β1 and polymerized actin were co-localized. In addition, protein tyrosine phosphorylation of both pp125<sup>FAK</sup> and paxillin occurred on infection of host cells with Sh. flexneri. Only soluble proteins were found to bind the integrin; how this leads to bacterial internalization remains to be shown (Watarai et al., 1996). Dehio et al. (1995) demonstrated that cortactin and pp60<sup>c-src</sup> (a cortactin tyrosine kinase) are enriched in membrane ruffles of host cells that engulf bacteria. They showed tyrosine phosphorylation of cortactin, a cytoskeleton-associated protein. These and other proteins recruited to the sites of Shigella entry into epithelial cells are components of focal adhesion plaques. In addition, Rho-specific inhibition was shown to impair Shigella entry into host cells and, unlike Salmonella, Ca<sup>2+</sup> influx is not required for Shigella entry (Menard et al., 1996).

Enteropathogenic E. coli (EPEC) are associated with characteristic attaching and effacing (AE) lesions on adhesion to host epithelial cells. AE lesions are characterized by disruption of microvilli at the site of attachment and rearrangement of host cytoskeleton beneath adherent bacteria into a pedestal-like structure containing cytoskeletal elements (actin filaments, α-actinin, ezrin, talin) and tyrosine-phosphorylated proteins. Mutants that do not cause AE lesions are non-invasive (Rosenshine & Finlay, 1993). A membrane-associated protein intimin (product of eaeA) is required for intimate association of bacteria with the host cell and for rearrangement of host-
cell cytoskeleton. However, signal transduction is generated by soluble factors (products of eaeB, fcm and class 5 loci) and the product of eaeB appears to be essential for activation of signalling pathways (Kenny & Finlay, 1995). Signal transduction events shown to occur on EPEC invasion include tyrosine phosphorylation of a 90 kDa protein of the host and elevation of intracellular levels of second messengers (IP3 and Ca2+; Kenny & Finlay, 1995; Rosenshine & Finlay, 1993).

Studies on the mucosal pathogens H. influenzae and N. meningitidis, which cause bacteraemia, also show differences in their interactions with epithelial and endothelial cells. Endothelial invasion by H. influenzae type b does not appear to be preceded by extensive plasma membrane activity in the host, and uptake involves processes arising in close vicinity of bacteria. The bacteria are surrounded in a manner that is reminiscent of coiling phagocytosis seen in professional phagocytes (Virji et al., 1991b; Fig. 3). Invasion by N. meningitidis, on the other hand, appears to induce relatively greater activity of the host cytoplasmic membrane and numerous processes can be seen by scanning electron microscopy (Fig. 3). The mechanisms of endothelial cell activation by these organisms are clearly distinct and probably utilize different signalling pathways, nevertheless involving the cytoskeleton, as evidenced by inhibition of invasion in the presence of cytochalasin D (Virji et al., 1991b, 1994a). In N. meningitidis, the outer-membrane protein (OMP) Opc mediates interactions with host-cell integrins by a bridging mechanism utilizing RGD-bearing serum proteins, and leads to cellular invasion (Virji et al., 1994a).

Localized actin polymerization and zipperring has been described for the mechanism of entry of Listeria monocytogenes and resembles that for Yersinia. In the latter case, the interactions with host cells occur via β1 integrins (see below). In contrast, L. monocytogenes uses E-cadherin receptors on host cells targeted via its 80 kDa surface protein, internalin (Mengaud et al., 1996). Targeting of distinct receptors by the two pathogens probably stimulates different signalling pathways, although, some events, such as tyrosine phosphorylation in host cells, appear to occur in both cases (Mengaud et al., 1996).

Invasion of host cells by the obligate intracellular protozoan parasite, Trypanosoma cruzi, the cause of Chaga's disease, occurs by an unusual mechanism and appears to be facilitated by disruption of host-cell actin microfilaments. Invasion can be enhanced by cytochalasin D treatment. In addition, pertussis toxin which acts on G-proteins (see below), as well as Ca2+ channel-blockers, inhibit Tryp. cruzi entry (Rodriguez et al., 1995). A recent study has implicated signalling pathways involving the TGF-β receptor for Tryp. cruzi invasion of epithelial cells (Ming et al., 1995).

**Second messengers**

Increases in second messengers such as Ca2+ and IP3 in host cells have been shown using peptides derived from Tryp. cruzi, and resulted in rearrangement of host-cell microfilaments (Rodriguez et al., 1995). However, increase in inositol phosphate on invasion of cultured epithelial cells with Helicobacter pylori, was not associated with redistribution of cytoskeletal proteins and was independent of bacterial adherence. This suggested a role for a soluble bacterial factor in stimulating this signalling pathway (Pucciarelli et al., 1995). Studies on Salmonella, EPEC and verocytotoxin-producing E. coli (VT) have also implicated stimulation of second messengers (Kenny & Finlay, 1995; Ismaili et al., 1995; Rosenshine & Finlay, 1993). EPEC stimulates host tyrosine kinases and its stimulation of IP3 is dependent on host phosphorylation. In contrast, cytosolic IP3 and Ca2+ elevation that occur on VTEC infection are independent of phosphorylation of host-cell proteins (Ismaili et al., 1995).
G-proteins. The Gram-positive bacterium *Streptococcus pneumoniae* fails to enter resting human cells, but invasion of host cells occurs, during inflammatory activation. This involves G-protein-coupled platelet-activating-factor receptor (PAF-R) expressed in activated cells. Bacterial cell-wall phosphorylcholine appears to mimic the ligand structure and interacts with PAF-R which is rapidly internalized after interaction with the ligand. However, normal signal transduction (activation of phospholipase C) did not occur on bacterial interaction with PAF-R (Cundell et al., 1995).

Some bacterial toxins enter eukaryotic cells and act directly on G-proteins. Cholera toxin (CT) and pertussis toxin (PT) ADP-ribosylate heterotrimeric G-proteins (Ga) of the subfamilies Gα and Gβγ. These modifications result in modulation of regulatory functions of the G-proteins and of pathways that include second messengers (Nurnberg et al., 1995).

Several members of the small G-proteins (RhoA, RhoB and RhoC but not Rac or Cdc42) act as substrates for ribosylation by *Clostridium botulinum* C3 toxin. Recently, a novel mechanism by which intracellularly acting bacterial toxins act on small G-proteins has been reported. *Clostridium difficile* toxins A and B, which enter eukaryotic cells by receptor mediated endocytosis, apparently monoglucosylate Rho, Rac and Cdc42. Other members of the Ras superfamily (Ras, Rab and Arf) are not affected. Since Rho proteins are involved in regulation of the actin cytoskeleton and involve second messengers, the effect of these toxins is characterized by destruction of cytoskeleton and morphological changes in the host cell (Aktories & Just, 1995).

**Protein tyrosine phosphatases**

YopH protein of *Yersinia* is a protein tyrosine phosphatase (PTPase) that appears to break down signal-transduction pathways in many cell types, including those of the immune system, enabling bacteria to avoid phagocytosis. PTPases are also encoded by viruses, for example, the VH1 gene in vaccinia virus encodes a phosphatase that is able to dephosphorylate phosphorylated serine, threonine and tyrosine (Guan & Dixon, 1993).

**Endotoxin and signal transduction**

LPS-mediated signal transduction mechanisms that result in the synthesis and release of inflammation mediators are not clearly defined at present. LPS clearance may involve signal-transduction-independent internalization. Two effector molecules, LBP and CD14, play important roles in LPS interactions with host cells, but LPS internalization may occur independently of these molecules. Diverging pathways of internalization and signal transduction have been examined recently (Gregner et al., 1995), and it is postulated that additional transducing molecules may activate signal-transduction pathways of the host. Membrane CD14 (mCD14 present on phagocytic cells is involved in surface location of LPS) is a glycosylphosphatidylinositol (GPI)-anchored molecule. In monocytes, cross-linking of mCD14 leads to the activation of Src family kinases (Seed, 1995). Epithelial and endothelial cells do not have mCD14. In these cells, LPS from certain Gram-negative bacteria can exert cytopathic effects. *In vitro* studies show that soluble serum CD14 (sCD14) is apparently required for toxic damage of bovine endothelial cells mediated by *H. influenzae* type b LPS (Patrick et al., 1992) or of human endothelial cells mediated by *N. meningitidis* LPS (K. L. R. Dunn & M. Virji, unpublished observations). An interesting aspect of the pathogenesis of these Gram-negative organisms in humans is that LPS-mediated toxicity for vascular endothelial cells is readily observed in the case of *N. meningitidis*, but not with *H. influenzae* type b. For example, purpuric skin lesions that arise as a result of endothelial necrosis and intravascular coagulation, are common in disseminated *N. meningitidis* infection, but rare during septicaemia caused by *H. influenzae* type b. Similar observations were made during *in vitro* studies; *N. meningitidis* but not *H. influenzae* type b cause LPS-dependent toxic damage to cultured human umbilical vein endothelial cells (Virji et al., 1991a, b). How *N. meningitidis* LPS/CD14 interactions with human endothelial cells differ from *H. influenzae* LPS/CD14 interactions to deliver signals that are lethal or benign is not known. One explanation lies in differences in the lipid A structures of the LPSs from these bacteria (Dunn et al., 1995). Lipid A has been shown to mimic ceramide structure and activate ceramide-activated protein kinases (CAPKs) (Joseph et al., 1994). This signalling pathway normally results in negative-regulation of cell growth and apoptosis (see above). Whether small differences in acylation of lipid A affect interactions with the hypothetical CAPK-activating intermediate remains to be shown.

In addition to CD14-mediated mechanisms, members of the integrin family, CR3 (αmβ2) and CR4 (αxβ2) may also interact with LPS. Signal transductions via CR4 receptors (as assessed by induction of NF-κB translocation) has been reported recently (Ingalls & Golenbock, 1995).

**Microbial interactions with adhesion molecules**

Adhesion molecules comprise a number of different families of molecules with a common overall structure which consists of a surface-exposed ligand-binding domain, a membrane-spanning region and a cytoplasmic tail. Salient structural features and a brief summary of functional aspects of distinct families are shown in Fig. 2. Specific targeting of different adhesion molecules by several micro-organisms is summarized in Table 1.

**Opportunism in microbial strategy of host receptor subversion: integrins as common targets**

Within the repertoire of cell-surface molecules and of adhesion receptors, integrins are a common target for pathogens, perhaps due to their ubiquitous presence on all cell types (Albeda & Buck, 1990). Also, engaging with these receptors provides the best strategy for manipulation of host-cell machinery for entry into cells and...
Table 2. Arginine-glycine-aspartic acid (RGD) in integrin targeting of microbes

<table>
<thead>
<tr>
<th>Microbe</th>
<th>Mechanism</th>
<th>Target receptor</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>RGD sequence in penton base</td>
<td>αβ3, αβ5, (α5β1)</td>
<td>Wickham et al. (1993)</td>
</tr>
<tr>
<td>Picornaviruses:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foot-and-mouth disease virus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Echovirus-22</td>
<td>RGD in capsid</td>
<td>αβ3</td>
<td>Mason et al. (1994)</td>
</tr>
<tr>
<td>Coxsackievirus-A9 (CAV-9)</td>
<td></td>
<td>αβ3</td>
<td></td>
</tr>
<tr>
<td>HIV</td>
<td>RGD sequence in Tat protein</td>
<td>α5β1, αvβ3</td>
<td>Barillari et al. (1993);</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Brake et al. (1990)</td>
</tr>
<tr>
<td>Bordetella pertussis</td>
<td>RGD sequence in FHA</td>
<td>CR3 (αmβ2)</td>
<td>Sandros &amp; Tuomanen (1993)</td>
</tr>
<tr>
<td></td>
<td>RGD sequence in pertactin</td>
<td>Epithelial integrins</td>
<td></td>
</tr>
<tr>
<td>Borrelia burgdorferi</td>
<td>RGD-dependent adhesion</td>
<td>αIibβ3</td>
<td>Leininger et al. (1992)</td>
</tr>
<tr>
<td>Neisseria meningitidis</td>
<td>Utilizes RGD-containing proteins</td>
<td>αvβ3 (α5β1)</td>
<td>Coburn et al. (1993)</td>
</tr>
<tr>
<td></td>
<td>via Opc</td>
<td></td>
<td>Virji et al. (1994a)</td>
</tr>
<tr>
<td>Streptomyces avidinii</td>
<td>RGD-like sequence (RYD) in streptavidin</td>
<td>α5β1 (α1Iibβ3)</td>
<td>Alon et al. (1993a, b)</td>
</tr>
<tr>
<td>Treponema pallidum,</td>
<td>Bind RGD of fibronectin</td>
<td>Cell-associated Fn</td>
<td>Thomas et al. (1985)</td>
</tr>
<tr>
<td>Treponema denticola,</td>
<td></td>
<td></td>
<td>Dawson &amp; Ellen (1990)</td>
</tr>
<tr>
<td>Trypanosoma cruzi</td>
<td></td>
<td></td>
<td>Ouassi et al. (1986)</td>
</tr>
<tr>
<td>Leishmania</td>
<td>RGD-like sequence (SRYD) in gp63</td>
<td>CR3 (αmβ2)</td>
<td>Russell &amp; Wright (1988);</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Soteriadou et al. (1992)</td>
</tr>
</tbody>
</table>

Opening up a reservoir of nutrition. In addition, entry into non-phagocytic cells may provide shelter from humoral as well as cellular immune mechanisms. Several distinct opportunistic mechanisms by which microbes subvert integrin receptors and associated signalling pathways may be defined, with molecular mimicry being the most common strategy.

**Ligand mimicry**

Direct interactions of micro-organisms with eukaryotic receptors often occur at the normal host ligand recognition site and may utilize the ligand recognition motif, for example the sequence RGD (Arg-Gly-Asp), a recognition sequence on many ligands of integrins. In addition, as is the case with natural integrin ligands, receptor specificity in microbes may be modified by RGD flanking sequences. Within the RGD motif, aspartic acid is important for recognition by integrins (Ruoslahti & Pierschbacher, 1987) and some integrins that do not recognize RGD sequence nevertheless require an acidic amino acid (Asp or Glu) for binding to ligands (Staunton et al., 1990; Isberg & Tran Van Nhieu, 1994). In some bacteria, e.g. Yersinia which binds multiple β1 integrins (the majority do not recognize RGD), aspartic acid has been shown to be essential in adhesion (Isberg & Tran Van Nhieu, 1994). Since some β1, β2 as well as β3/β5 integrins may bind RGD, expression of this sequence on microbial ligands imparts the potential to interact with multiple integrins.

**Microbes that possess RGD or RGD-like sequences.** Two of the virulence-associated proteins of Bordetella pertussis (filamentous haemagglutinin and pertactin), contain the RGD sequence which is involved host-cell interactions (see below). Several viruses contain the RGD sequence (Table 2). Other microbes contain RGD-like sequences. For example, streptavidin (a tetrameric analogue of avidin) which is secreted by Streptomyces avidinii, has considerable homology to the RGD-containing integrin-binding domain of fibronectin and may bind to cells via the motif RYD. It interacts with the specific fibronectin receptor, α5β1. Its specificity for α5β1 may be acquired from the RYD flanking sequences (Alon et al., 1993a, b).

Leishmanial surface-located protease, gp63, is a fibronectin-like molecule and reacts with anti-fibronectin antibodies (Rizvi et al., 1988). Gp63 does not contain an RGD sequence, but as in streptavidin, a related sequence (SRYD) replaces RGD functionally in recognizing integrins on host macrophages (Soteriadou et al., 1992). The major receptor for gp63 has been shown to be CR3 (Russell & Wright, 1988).

**Pseudoligand mimicry, sandwich mechanism: microbes that bind RGD/RGD-containing proteins or other adhesion molecules.** Microbial adhesion to integrins may also occur by a bridging or sandwich mechanism involving natural ligands of integrin receptors such as extracellular matrix proteins. In the case of the leucocyte integrin CR3 (αmβ2), the coating of microbes by the ligand, complement component C3bi, renders the microbe resistant to in-
tracellular killing (see below). Not all sandwich adhesion results in ligand/microbe internalization; for example, coating of the bacterial surface with fibronectin may result, in some cases, in extracellular location only (Isberg, 1991).

Several Gram-positive bacteria (staphylococci, streptococci, lactobacilli) interact with fibronectin, collagen, laminin, vitronectin, thrombospondin, elastin, bone sialoprotein and fibrinogen (Patti et al., 1994). In addition, many Gram-negative bacterial fimbriae adhere to ECM components via protein–protein or protein–carbohydrate interactions. Type I fimbriae of E. coli and Salmonella have been shown to bind mannoside chains of matrix proteins (Patti et al., 1994). Type I fimbriae of E. coli also interact with mannosyl residues on CD11/CD18 (αxβ) integrins structurally, and antibodies raised against mammalian integrins specifically react with microbial integrin-like structures (M. Virji, 1993).

The spirochaetes Treponema pallidum (causing syphilis) and Treponema denticola (an oral commensal) adhere to fibronectin via its RGDs sequence. Such integrin-like interactions with fibronectin may allow them to adhere to host cells via surface-located ligands. It is suggested that treponemes bind to one of the two RGD sequences (in fibronectin dimer) not occupied by host-cell fibronectin receptor (Thomas et al., 1985; Dawson & Ellen, 1990). Treponema cruzi may also use a similar mechanism for host-cell adhesion (Ouaissi et al., 1986).

Fibronectin is apparently a common target utilized by many bacteria, as well as by yeasts. Whilst adhesion via receptor ligands may not always result in microbial uptake (Isberg, 1991), affinity of ligand–receptor binding and receptor density are among the factors that determine the fate of the adherent microbe (see below).

**Receptor mimicry: microbial mimicry of the integrin structure**

Some micro-organisms express proteins that resemble integrins structurally, and antibodies raised against mammalian integrins specifically react with microbial integrin-like structures.

Mycobacterium avium–intracellulare is ubiquitous in the environment but is able to act as an intracellular pathogen. Disease due to this organism is rare and usually occurs in immunocompromised hosts, and is responsible for the highest incidence of disseminated bacterial infection in patients with AIDS. Integrin β1-reactive antibodies cross-react with bacterial extracts. Also, mycobacteria bind avidly to laminin, collagen I and fibronectin via a β1-integrin-like structure and the interactions are cation-dependent (Rao et al., 1992). Mycobacterial integrin can interact also with RGD on the Tat protein of human immunodeficiency virus (HIV) (Denis, 1994). It is suggested that the interaction between Tat, present in body fluids of HIV-infected patients, and this opportunistic pathogen, may contribute to the acute susceptibility of AIDS patients to mycobacterial infection. Candida, a normal commensal yeast of the intestinal tract, becomes pathogenic in immunocompromised patients, neonates and diabetics. The presence of an antigenically, structurally and functionally similar protein to the αβ subunit of the CD18 (β2) integrins CR3 (αm) and CR4 (αx) has been demonstrated in C. albicans (Hostetter, 1994). The yeasts adhere to host epithelial cells via RGD-dependent mechanisms and the ligands on the host epithelial cells are reported to be surface-located integrin ligands, C3bi for C. albicans and fibronectin for Candida tropicalis. Two important differences between Candida integrins and mammalian integrins are that the β1 subunit has not been demonstrated in Candida and binding of the yeast integrin to C3bi is cation-independent. However, *Candida* may express β1-like integrins and may bind to fibronectin via this molecule (Hostetter, 1994; Klotz et al., 1992).

**Complex mimicry**

**Multiple mimicry in Bordetella.** The most striking example of the mimicry of host cellular recognition molecules is seen in *Bordetella pertussis* filamentous haemagglutinin, FHA (Sandros & Tuomanen, 1993). This protein contains several distinct sites involved in interactions with glycoconjugates, heparin and CR3 (an RGD motif). It also contains sequence similarities to endothelial ligands, factor X and to C3bi, and represents mimicry of multiple ligands recognized by the leucocyte integrin CR3 (αβ2) (Sandros & Tuomanen, 1993). In addition to FHA, *Bord. pertussis* expresses other virulence-related proteins which include pertactin and pertussis toxin. Pertactin also contains an RGD sequence and is implicated in adhesion to epithelial cell integrins. It is suggested that flanking sequences of RGD in FHA and pertactin determine the receptor- and tissue-specificity of interactions of the microbe via RGD (Leininger et al., 1992). Pertussis toxin subunits S2 and S3 share similarities with selectins.

**Other mechanisms**

In contrast to the above examples, echovirus does not appear to use mimicry as a mechanism of interacting with the integrin α2β1. Interactions of the virus and the integrin ligands (extracellular matrix proteins) occur by distinct mechanisms, and antibodies that block binding of the virus do not inhibit integrin binding to collagen or laminin. This interaction also results in viral internalization (Bergelson et al., 1992).

**Adhesion vs integrin-mediated invasion via integrins**

Yersinia, with its multiple virulence mechanisms, provides an interesting example of complex adhesion/invasion...
events manipulated via encounter with cellular integrins. Chromosomally coded invasin interacts with several β1 integrins and this results in bacterial internalization (Isberg, 1991). The factors that determine uptake of Yersinia via β1 integrins (which primarily mediate adherence to their ECM ligands) were investigated by Tran Van Nhieu & Isberg (1993). One of the mechanisms leading to invasion may be multiple occupancy on the receptor [as described for Leishmania interactions with CR3 via ligands gp63 and lipopolysaccharide (LPS), which results in internalization; Talamas-Rohana et al., 1990]. This does not occur in the case of Yersinia invasin/β1 interactions since a small region of invasin mediates both binding and invasion (Isberg, 1991). However, unlike the natural ligand, invasin engages with β1 integrins via high-affinity interactions. This may allow efficient competition with other ligands and result in 'zippering' - a process by which the host cell forms a series of contacts over the surface of the micro-organism, leading to uptake (Tran Van Nhieu & Isberg, 1993; Isberg, 1991). The site of attachment to integrins appears to be unimportant; high-affinity interactions to any site on α5β1 integrins resulted in internalization. Additionally, receptor clustering achieved by cross-linking of ligands was a requirement for signal leading to uptake (Tran Van Nhieu & Isberg, 1993). It is also suggested that high receptor density may achieve the same end. Thus, up-regulation of integrin receptors during many viral and other (e.g. malarial) diseases may help augment widespread invasion. In the absence of the invasin protein, YadA can mediate interactions and invasion also via β1 integrins, and this may occur via a bridging molecule, such as fibronectin, that binds to YadA. Interestingly, internalization by invasion proteins of Yersinia may be inhibited by other plasmid-encoded proteins, Yops, which appear to act on host cytoskeletal proteins to dephosphorylate. Thus Yersinia can bind integrins which can lead to cellular invasion, but also have the capacity to modulate subsequent events by interfering with signal transduction via accessory proteins (Bliska et al., 1993).

The immunoglobulin superfamily

The Ig superfamily includes a diverse array of receptors defined by the presence of one or more copies of the Ig fold, a compact structure of 60–100 amino acids arranged in facing β-sheet structures. The receptors are involved in cell-adhesion events, including cell–cell interactions and antigen recognition. Besides being utilized by several bacteria and parasites, the members of the Ig family are targets of several viruses that belong, in the main, to the picornaviridae (Crowell & Tomko, 1994) (Table 1). Several picornaviruses possess a 'canyon' structure in their capsid which is implicated in adhesion to particular Ig folds of the receptor.

Poliovirus receptor (PVR)

Studies on many viral systems particularly implicate cellular receptors in viral tissue tropism. Poliovirus infection of a limited number of host tissues exemplifies this. The receptor for the virus (PVR) has been shown to be structurally related to members of the Ig superfamily (Mendelsohn et al., 1989), its function is not known. Although PVR is required for poliovirus infection, tissue tropism of the virus may depend on another adhesion molecule, the lymphocyte homing receptor (CD44, Shepley & Racaniello, 1994). Tissue specific glycosylation of the receptor isoforms may also influence PVR interactions with the virus and determine its tissue tropism (Bernhardt et al., 1994).

NCAM

Molecular mimicry of the adhesion molecule NCAM by certain microbes may involve an additional strategy, distinct from those discussed above. NCAM is a heavily sialylated molecule and neonatal forms of NCAM contain more sialic acid groups than adult NCAM. Bacteria causing meningitis in neonates, such as E. coli K1 and N. meningitidis group B, contain α2,8-linked polysialic acid capsules that resemble structures found in neonates but not in adults. Antibodies to meningococcal capsule have been shown to react specifically with embryonic but not adult NCAM. The success of these microbes in brain tissue colonization may be attributed to their ability to escape host immune detection by mimicking surface glycans on adhesion molecules of the target tissue (Finne et al., 1983, 1987; Rougon et al., 1986).

CD66 (CEA)

The carcinoembryonic antigen (CEA) family (also known as CD66) includes clinically important tumour markers, such as the CEA, which is up-regulated in epithelial carcinomas. The CEA family also includes cross-reacting antigens, e.g. non-specific cross-reacting antigens (NCAs), biliary glycoproteins (BGP)s and pregnancy-specific glycoproteins (PSGs). The members of the CEA family share antigenic determinants and show high levels of similarity in amino acid sequences (Watt et al., 1994). The CEA family are targeted by E. coli type 1 fimbriae (Sauter et al., 1993) as well as by Opa proteins of both N. meningitidis and N. gonorrhoeae (M. Virji and others, unpublished, see below).

Multiple receptors of Plasmodium falciparum

Pathology in malaria is associated with cytoadherence of paracytosed erythrocytes. In vitro studies have shown that P. falciparum-infected erythrocytes interact with several molecules on endothelial cells, including thrombomodulin, CD36, ICAM-1, VCAM-1 and ELAM-1, and also adhere to human monocytes and platelets via CD36 (Berendt et al., 1989; Pasloske & Howard, 1994). Parasite clones vary in adherence to these receptors, but at least some clones have the potential to interact strongly with multiple receptors (Pasloske & Howard, 1994).
The selectins

E-, P- and L-selectins are involved in inflammatory responses and exhibit lectin-like activity used in functional mimicry by some microbes. The pertussis toxin (PT) produced by *Bord. pertussis* is a hexameric protein with carbohydrate-binding property and recognizes lactosyl-ceramide and gangliosides from epithelial-cell cilia and macrophages. The carbohydrate-recognition region of PT is strikingly similar to the region 15–46 of E- and P-selectins. Moreover, PT subunits S2 and S3 with the carbohydrate-binding domains inhibit neutrophil adhesion to purified selectins, leucocyte adhesion to endothelial cells, and are anti-inflammatory in an animal model for meningitis (Sandros & Tuomanen, 1993). In vitro studies suggest that PT subunits S2 and S3 up-regulate CR3 in macrophages, in a manner similar to that observed with selectins. CR3 integrin is also the receptor for *Bord. pertussis* FHA. Thus, PT and FHA function as cooperative adhesins (Sandros & Tuomanen, 1993).

The cadherins

The calcium-dependent cadherin adhesion molecules involved in intercellular interactions are utilized by *Sh. flexneri* and *L. monocytogenes*. Sansonetti et al. (1994) have shown that intercellular spread of *Sh. flexneri* requires the expression of cadherins that maintain the intercellular junctional integrity within intact epithelium. Cadherin expression appears to be required for efficient bacterial anchorage to the internal face of the cytoplasmic membrane of infected cells. In addition, it is required for homotypic interactions between the surface of cell protrusions (containing bacteria) and that of an adjacent cell to facilitate internalization by that cell (Sansonetti et al., 1994; Menard et al., 1996). *L. monocytogenes* surface-located protein, internalin, has been shown to target E-cadherin on epithelial cells for cellular entry (Mengaud et al., 1996).

Adhesion receptor modulation in pathogenesis

*Borrelia burgdorferi* up-regulates adhesion molecules such as E-selectin, P-selectin, ICAM-1 and VCAM-1 on mouse endothelial cells in vitro, and these may play an important role in the pathogenesis of *Borrelia* infections (Boggemeyer et al., 1994). Such increased expression may conceivably render host cells more susceptible to invasion by other pathogens utilizing these receptors, since receptor density may play a role in microbial location in or out of the host cell (discussed above). Certain microbes appear to induce their own receptors by supplementary mechanisms. *Bord. pertussis* appears to up-regulate CR3 via FHA interactions involving leucocyte signal-transduction complex comprising a B3 integrin and CD47 (Ishibashi et al., 1994), as well as via selectin-like function of PT (Sandros & Tuomanen, 1993). In malarial infection, up-regulation of receptors has also been observed. Brain endothelium from patients dying of malaria expressed several of the implicated malarial receptors (CD36, ICAM-1, ELAM-1 and VCAM-1; up-regulated or induced), whereas brain tissue from non-infected patients did not show up-regulation of these adhesion molecules (Pasloske & Howard, 1994). The increased expression of some of these receptors may result from increased levels of TNFα observed in malaria patients (Pasloske & Howard, 1994).

Respiratory syncytial virus down-regulates adhesion receptors such as LFA-1 and ICAM-1 on mononuclear cells (Salkind et al., 1991). In contrast, parainfluenza virus type 2 up-regulates the expression of ICAM-1 and other receptors on human tracheal epithelial cells (Tosi et al., 1992), so that these cells are able to bind increased numbers of neutrophils. Modulation of several adhesion molecules has been demonstrated in HIV disease and involves T-cells as well as phagocytic cells (Weeks et al., 1991).

*N. meningitidis* interactions with human cells – an example of the role of multiple adhesive ligands and molecular mimicry in pathogenesis

**Interactions via the OMP Opc**

*N. meningitidis* resides in the nasopharynx of its human host and further sequestration occurs from this primary and specific site of colonization. Factors necessary for colonization and for epithelial invasion that lead to serious pathogenic conditions remain to be described fully. Amongst the virulence factors elaborated by the organism are pili (fimbriae), filamentous multimeric protein structures, and OMPs that include the proteins Opa and Opc. Opc, a basic 28 kDa protein, appears to have the capacity to interact with multiple extracellular matrix components and serum proteins (Virji et al., 1994b), giving the organism the potential to interact with several different integrins by bridging via their respective ligands. This could be an effective strategy for cellular invasion and for adhesion to substrata of damaged mucosa, as well as for penetrating deeper tissues after cellular invasion. Indeed, Opc mediates cellular invasion of cultured epithelial and endothelial cells (Virji et al., 1992). These interactions can be inhibited by monoclonal antibodies against Opc which appears to be the major requirement on bacteria for this interaction. However, cloned Opc does not confer invasive properties on E. coli, even though the protein is surface-expressed and is immunologically similar to that on *N. meningitidis*. This suggests that additional bacterial factors may be required in host-cell interactions mediated by Opc. It is also possible that the level of Opc expressed by *E. coli* is not optimum since efficient interactions of *N. meningitidis* via Opc require the protein to be expressed at a high density on the bacterial surface (Virji et al., 1995).

**RGD-dependent cellular invasion mediated by Opc**

Studies using cultured human endothelial cells have shown that interactions of Opc-expressing *N. meningitidis*
Microbial utilization of human signalling molecules

Fig. 4. Immunofluorescence micrograph showing RGD-dependent interactions of N. meningitidis with the apical avβ3 integrins of confluent endothelial cells in culture. (a) Control monolayers infected with Opc-expressing meningococci. Bacteria were stained with monoclonal antibody SM82 directed against lipopolysaccharide and rhodamine-conjugated second antibody. (b) RGD-mediated inhibition of interactions of Opc-expressing N. meningitidis (RGD present in serum-supplemented infection medium). RGE-containing peptides were not inhibitory (data not shown). (c) Inhibition of bacterial interactions in the presence of antibody (LM609) against vitronectin receptor (Virji et al., 1994a).

Fig. 5. Mechanism of meningococcal entry into human endothelial cells.

with the apical surface of polarized host cells require serum-derived factors. These factors appear to be RGD-containing proteins, and RGDS, but not RGES, peptides inhibit bacterial invasion of human endothelial cells. Moreover, antibodies against vitronectin receptor (avβ3, VNR) and fibronectin receptor (α5β1, FNR) inhibit adherence and invasion. VNR appears to be the major receptor involved in serum-dependent apical interactions of N. meningitidis, but FNR may also be involved (Virji et al., 1994a) (Figs 4, 5).

Although pseudoligand mimicry appears to be utilized by Opc-expressing N. meningitidis, it is possible that for signalling further factors are involved in the interactions via the VNR. CR3 has been shown to interact simultaneously with C3bi-coated particles and with microbial glycolipids at distinct sites. Indeed, VNR also exhibits binding sites for ganglioside GD2 (Cheresh et al., 1987). Gangliosides and LPS share structural similarities in that both are amphipathic with a strongly anionic hydrophilic group and some manner of LPS interaction with vitronectin receptor may be an additional factor required. This is at present a speculation and there is no evidence to support this hypothesis.

Two other lines of evidence support the proposed involvement of integrins, especially of VNR. Capillary endothelial cells derived from human foreskin were shown to be lacking reactivity with monoclonal antibodies against VNR, and indeed are one of the few cells not to express av (Albelda & Buck, 1990). These cells failed to support Opc-mediated adhesion or invasion. Also, treatment of human umbilical vein endothelial cells with certain cytokines that modulate integrin expression, also affects bacterial interactions mediated by Opc (unpublished observations).

An interesting feature of the Opc interaction is the requirement for host cytoskeletal function. Attempts to inhibit host-cell invasion by the use of cytochalasin D resulted in inhibition not only of invasion but also of total cell association. This observation is in contrast to cell adhesion mediated by N. meningitidis Opa proteins which increases in the presence of cytochalasin D, although invasion is inhibited (Virji et al., 1994a). It has been suggested that efficient bacterial internalization requires direct adherence of bacteria to host receptors (Isberg, 1991), and fibronectin-dependent sandwich interactions do not mediate uptake unless receptors are disengaged.
from matrix binding. Affinity of ligand–receptor interactions may also determine attachment/uptake via the same receptor (Isberg, 1991). In the case of N. meningitidis, serum-protein-dependent Opc interactions results in invasion, suggesting that these are high-affinity interactions with the receptors.

**Pilus-dependent interactions and up-regulation of invasion of human endothelial cells mediated by Opc**

Meningococcal strains isolated from blood or CNS are invariably capsulate and many, but not all nasopharyngeal isolates also possess capsule. Capsular polysaccharide which coats the outer membrane of the bacteria is protective against host’s immune mechanisms; for example, it imparts resistance to phagocytosis (McNeil et al., 1994). Most capsule meningococci isolated from patients also express pili. These proteins are effective adhesins in capsule bacteria since unlike other outer-membrane adhesins, pili traverse the capsule and are largely unaffected by it (Virji et al., 1991a, 1995). Pili are used by capsule organisms to adhere specifically to human epithelial and endothelial cells. In addition, in capsule-deficient bacteria that are often isolated from the nasopharynx, pili may also become accessory proteins to other outer-membrane adhesins and invasins such as Opc described above. In recent studies, a modulatory role of pili in cellular invasion mediated via integrins was demonstrated (Virji et al., 1995). Pili also augment cytopathic damage of N. meningitidis (Dunn et al., 1995). Pili have been shown to be post-translationally modified and in particular contain glycosyl structures of unique composition (Virji et al., 1993; Stimson et al., 1995). Whether these structures have significance in host interactions, via lectin-like receptors, remains to be investigated.

**Opa proteins and their targeting of CD66**

Opa proteins of N. meningitidis and the related pathogen N. gonorrhoeae are a family of structurally related proteins (Cannon, 1994). Most Opa proteins have been shown to mediate interactions with neutrophils and some also interact with epithelial cells. Recent studies using transfected COS cells expressing several adhesion receptors have shown that most Opa proteins of numerous strains of both N. meningitidis and N. gonorrhoeae interact specifically with CD66-expressing cells. The adhesion was to the N-terminal domain of the molecule which is largely conserved between different members of CD66. An antibody against the N-terminal domain inhibited Opa-expressing bacterial interactions with human PMN and some epithelial cells known to express CD66. Since the antibody recognizes distinct members of the CD66 family (Watt et al., 1994), Opa proteins may target distinct CD66 molecules via the largely conserved N-domain and thus interact with diverse cells via the CD66 family of molecules (M. Virji and others, unpublished).

**Clinical considerations**

**Clinical conditions involving adhesion molecules, modulation of microbial infection and therapeutic considerations**

Leucocyte-adhesion-deficiency syndrome (LAD) is an inherited disorder involving the β2 chain (CD18) of the leucocyte cell adhesion molecules LFA-1, CR3 and CR4, and results in a low number or absence of normal receptors on the cell surface (Arnaout, 1990). The genetic defect can be corrected in vitro by transfection using normal CD18 coding sequences. Patients with LAD develop severe bacterial infections involving oral, respiratory and urogenital mucosa, as well as the skin and the intestine. The infections seem to result from impaired adhesion-dependent functions such as chemotaxis, aggregation and CR3-dependent phagocytosis (Hamacher & Schaberg, 1994). A similar situation has also been observed in the rare condition of impairment in the expression of sialyl-Lewis^x^ on neutrophils, a binding site for endothelial E-selectin (Etzioni et al., 1992). These conditions emphasize the importance of CD18 integrins and selectins in host defence against microbes.

**The potential for intervention in microbial diseases involving adhesion receptors**

With the aid of monoclonal antibodies or receptor analogues, it is possible to interfere with specific receptor functions that involve interactions with microbial ligands. The use of such a therapy has obvious associated implications of side-effects since adhesion molecules have central roles in host cellular functions. Receptor blockade may be useful under extreme circumstances, and specific ligands such as RGD-bearing proteins or peptides have also been considered appropriate for use in such intervention. However, RGD peptides may cause endothelium-dependent aortic relaxation (Klotz et al., 1992) which could lead to exposure of ECM and increased adherence of some microbes. The use of RGD in experimental candidiasis reported decreased yeast load in tissues. A peptide (PepTite-2000) with an amino acid sequence modelled after RGD cell-binding domain of fibronectin was used to modulate hematogenous candidal infections in a rabbit model of infection. The reduction in tissue infiltration is presumed to result from candidal integrins blocked by the fibronectin mimic (Klotz et al., 1992).

A possible therapeutic potential for anti-adhesion-molecule antibodies has been suggested for management of bacterial meningitis, with intervention targeted at the anti-inflammatory role of antibodies against CD18 (β2) integrins to inhibit the recruitment of white blood cells into the subarachnoid space (Saez-Llorens et al., 1991). The adverse pathophysiological consequences resulting from infiltration were reduced by a combined therapy of dexamethasone to diminish overproduction of cytokines in response to bacterial stimulus and anti-integrin antibodies (Saez-Llorens et al., 1991). Anti-CD18 (CR3) antibodies have also been effective in increasing survival...
of experimental mice after endotoxin challenge, and may be of potential therapeutic value against Gram-negative sepsis and shock (Burch et al., 1993).

Concluding remarks
Mimicry emerges as a common strategy employed by numerous pathogens in subverting host cellular signalling pathways for invasion. However, invading microbes are constantly subject to host immune surveillance and avoidance of detection by neutralizing antibodies is an additional necessity for survival. To this end, antigenic variation occurs in many virulence-associated ligands. However, receptor-binding domains need to be conserved, and some microbes have evolved specific structures to protect these sites from neutralizing antibodies. Studies on viruses have provided examples of distinct mechanisms. The canyon structure of picornaviruses provides physical isolation of a conserved binding site. It allows the single Ig domain of the receptor to reach the binding site, but is narrow enough to exclude paired Ig domains of the antibody molecules (Crowell & Tomko, 1994). In contrast, in foot-and-mouth disease virus, the receptor-binding motif RGD is located within a flexible and a highly variable peptide loop protruding from the surface of the virus. The structural flexibility may prevent formation of epitopes recognizable by the host immune system. The ordered structure required for receptor binding may be induced by environmental factors (e.g. pH) at the cell surface or conformational changes induced by the receptor itself (Logan et al., 1993; Chapman & Rossmann, 1993).

Antigenic variation is common in bacteria such as N. meningitidis, but not all ligands exhibit a high level of variation, for example, Opc structure is largely conserved. However, Opc and several other meningococcal virulence factors are subject to phase variation—which assists immune avoidance. This method may be adopted for ligands with functional roles early in disease prior to antibody formation. Additionally, on/off phase variation has the potential to regenerate phenotypes in micro-environments that may be inaccessible to antibodies.

Another widely used mechanism of immune avoidance is host mimicry. Thus, mimicry in different forms is utilized by numerous microbes for dual purposes of avoidance of detection and for subversion of host cellular signalling pathways for invasion. Also, in many cases, factors that determine the natural ligand-specificity also determine tropism of the pathogen. Therefore, studies on microbe-host interactions may lead, in the future, also to discoveries of new receptors (as exemplified by the discovery of the novel molecule PVR), receptor functions and mechanisms of eukaryotic signalling pathways.

References


promastigotes binds to members of the CR3, p150,95 and LFA-1 family of leukocyte integrins. *J Immunol* 144, 4817–4824.


