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A complex interplay between the extracellular matrix and the innate immune response to microbial pathogens

Short title: The extracellular matrix and infection

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Summary

The role of the host extracellular matrix (ECM) in infection tends to be neglected. However, the complex interactions between invading pathogens, host tissues and immune cells occur in the context of the ECM. On the pathogen side, a variety of surface and secreted molecules, including microbial surface components recognizing adhesive matrix molecules (MSCRAMM) and tissue degrading enzymes, are employed that interact with different ECM proteins in order to effectively establish an infection at specific sites. Microbial pathogens can also hijack or misuse host proteolytic systems to modify the ECM, evade immune responses or process biologically active molecules such as cell surface receptors and cytokines that direct cell behaviour and immune defense. On the host side, the ECM composition and three-dimensional ultrastructure undergo significant modifications, which have a profound impact on the specific signals that the ECM conveys to immune cells at the forefront of infection. Unexpectedly, activated immune cells participate in the remodelling of the local ECM by synthesizing ECM glycoproteins, proteoglycans and collagen molecules. The close interplay between the ECM and the innate immune response to microbial pathogens ultimately affects the outcome of infection. This review explores and discusses recent data that implicates an active role for the ECM in the immune response to infection, encompassing antimicrobial activities, microbial recognition, macrophage activation, phagocytosis, leukocyte population balance and transcriptional and post-transcriptional regulation of inflammatory networks, and may foster novel antimicrobial approaches.

Introduction

To successfully establish an infectious disease, most pathogenic bacteria adhere to the host, invade underlying tissues, multiply and grow while evading or overcoming host immune defences (1). The underlying host-pathogen interactions are remarkably specific and involve a variety of substrates within the host. The interactions of pathogens with eukaryotic cells

has been intensively studied in microbiology, immunology, cell biology, genetics and ecology research. Less attention has been given to the host extracellular matrix (ECM). However, the individual components of the ECM are substrates to which pathogens can directly bind to or degrade, facilitating adhesion and penetration into the host; evidence for this started to accumulate 40 years ago (2, 3). Furthermore, the ECM is a fundamental component of the host cellular microenvironment where most of the events leading to infection, disease and tissue repair take place and is also a reservoir of diverse and tissue-specific signals that feed into immunological pathways (4, 5). Recently, evidence has emerged that the ECM conveys specific signals to cells thereby modulating essential immune functions, immune cell migration into and within infected tissues and immune cell activation, proliferation and differentiation. Moreover, the ECM composition undergoes significant alterations during infection and, notably, specific immune cell types appear to contribute to that. Thus, regulatory circuits closely link the ECM and the immune system.

Important questions to emerge from these studies include i) does the ECM play an active role in infection rather than simply providing a scaffold for bacterial adhesion or being a barrier to breach; ii) which ECM-pathogen interactions significantly impact the ability of pathogens to colonise and invade host tissues, and/or bypass host defences, and influence how host cells respond to pathogens; and iii) can we interfere with these interactions to develop new antimicrobial strategies or improve existing ones. Here, we briefly review the ECM and its interactions with microbial pathogens, and discuss the evidence of a direct implication of the ECM in the immune response to infection.

The ECM: an immunological perspective

Most infections and the resulting immune responses are tissue-specific. Each tissue has distinct ECM signatures which arise from complex and dynamic combinations of up to ~300 different proteins in varying concentrations and geometries within the three-dimensional extracellular space. This ever increasing number of proteins which contribute to matrices (the "matrisome") include approximately 43 collagen subunits, 36 proteoglycans and 200 glycoproteins (6). ECM molecular multiplicity and complexity are amplified by posttranslational modifications, which can unveil cryptic epitopes or generate new ones capable of triggering immune reactions, and alternative splicing, which generates several isoforms with distinct functions (7, 8). For instance, laminins α4 and α5 isoforms in basement membranes contribute to immune cell subtype selectivity during leukocyte recruitment to sites of inflammation (9, 10).

Interstitial ECMs, loose fibril-like matrices that fill the tissue stroma, and basement membranes, laminar sheets that anchor cell layers to underlying tissues, are basic forms of ECM. Additionally, specialised reticular fibre networks combining features of these two ECM structures are found in secondary lymphoid organs (11, 12).

The ECM is a highly dynamic yet strictly regulated tissue component. Its composition and normal function are determined and maintained by a fine balance between ECM synthesis, orchestrated by cytokines such as transforming growth factor-β (TGF-β), and turnover, accomplished by matrix metalloproteinases (MMPs), a disintegrin and metalloproteinases (ADAMs) and a disintegrin and metalloproteinases with thrombospondin motifs (ADAMTSs), whose activity is controlled by tissue inhibitors of metalloproteinases (TIMPs) (13). During injury and infection, these enzymes are produced by activated immune cells such as

monocytes/macrophages and promote immune cell migration into sites of infection and tissue damage and affect their ability to mount inflammatory responses (14).

The central dogma of matrix biology describes how the ECM provides structural support for cells and contributes to the unique structure of a tissue. However, this is only one function of the ECM. Host immune responses are carried out in the context of the ECM therefore when immune cells contact the ECM, they receive vital instructions for their survival, proliferation, differentiation and activation, in addition to support for adhesion and guidance for migration. Several mechanisms are responsible for the communication between the ECM and cells. One such mechanism is signalling through cell surface adhesion molecules and receptors, including integrins and discoidin-domain receptors (DDRs) (reviewed in (15, 16)). Others involve binding, storage, activation and release of secreted molecules with potent immunomodulatory activity, including cytokines, chemokines and growth factors (17, 18). For instance, TGF-β has been shown *in vitro* and *in vivo* to affect activation, proliferation and differentiation of most immune cell types, modulating nearly all stages of the immune response (19). Moreover, bioactive ECM fragments generated by tissue destruction (e.g. hyaluronan and heparan sulfate oligosaccharides) and ECM molecules whose expression is specifically induced upon tissue injury (e.g. fibronectin isoforms containing extra domain A (FN-EDA), versican and biglycan) promote inflammation by inducing pro-inflammatory gene expression or exhibiting chemoattractant properties (reviewed in (20, 21)). These molecules form a class of endogenous damage-associated molecular patterns (DAMPs) (22), which, by activating pattern recognition receptors (PRRs) such as toll-like receptors (TLRs) in immune cells (e.g. macrophages and dendritic cells) and non-immune cells (e.g. fibroblasts and epithelial cells) alert the immune system to tissue damage and infection that initiates pathogen clearance, but also tissue repair (23). Thus, the ECM and the immune system are intertwined: signals from the ECM help coordinate immune responses and, in turn, immune cells promote ECM repair and regeneration through the release of cytokines such as tumour

necrosis factor (TNF), interferon-γ (IFN-γ) and TGF-β, which regulate the expression of many ECM molecules.

ECM-pathogen interactions

A tale of adhesion and colonization of the host

Tissue adherence represents the first essential mechanism that bacteria adopt to colonize the host. Failure to do so results in the organism being removed by physiological cleansing systems at sites of entry. The surface of host cells and the ECM are negatively charged and microbes employ a number of physico-chemical forces to overcome these repulsive forces and establish interactions between a microbial ligand or "adhesin" and a complementary molecule or "receptor" on the host tissue. These interactions are highly specific, contributing to tissue tropism, species specificity and genetic specificity within a species. Bacterial adhesins are components of capsules, cell walls, pili or fimbriae, and those that bind to ECM components are called microbial surface components recognizing adhesive matrix molecules (MSCRAMM). Host receptors are usually glycoproteins found on the cell membrane and ECM components (24).

The binding of microbial pathogens to specific ECM proteins has been extensively reviewed elsewhere (1, 3, 25, 26) and will be briefly discussed here. The first report of a host ECM-pathogen interaction was published in 1978 by Kuusela *et al*. who studied the binding of *Staphylococcus aureus* to fibronectin (2) and, a few years later, revealed two separate binding sites on fibronectin, in the N- and C-terminus respectively (27). Other groups further characterised the interaction of this ubiquitous and promiscuous ECM glycoprotein with *S. aureus* and *Streptococcus pyogenes*, which has a remarkably large number of fibronectin

binding adhesins (28-33). Several other ECM proteins such as laminin, collagen, heparan and chondroitin sulphate, vitronectin, thrombospondin, elastin, bone sialoprotein and tenascin-C, have since been implicated in specific interactions with pathogenic bacteria ((26, 34); Table 1).

Early studies were mostly limited by the use of binding assays, involving bacterial cells or recombinant adhesins and ECM molecules, and inhibition experiments to demonstrate these interactions. Cutting-edge technologies, including live cell imaging and particle-tracking methods, have now started to reveal the implications of these interactions in pathogenesis. For example, Niddam *et al*. showed that the Lyme disease spirochete *Borrelia burgdorferi* exploits fibronectin to interact with vascular surfaces under physiological shear stress. Specifically, it recruits and induces polymerization of soluble plasma fibronectin that strengthens and stabilizes bacterial interactions with endothelia by a catch-bond mechanism (35).

Breaking down barriers to invade the host

Having colonized the host, most pathogens need to invade tissues in order to cause disease. This requires the breakdown of primary and/or secondary defences of the host, involving the crossing of basement membranes and interstitial matrices. Pathogens have developed distinct ways to modify the ECM (1, 36). They can directly degrade ECM components using “invasins” or bacterial tissue degrading enzymes such as hyaluronidases and collagenases, causing local tissue damage. Specific examples of these interactions are reported in Table 2 and reviewed elsewhere (26, 36). Degradation of the ECM not only facilitates the spread of pathogens, but can also favour tissue necrosis, bacterial toxin diffusion and host cell adhesion, migration and survival. Pathogens can also indirectly modify the ECM by altering the synthesis and turnover of ECM components by host cells in

response to their presence (discussed later in this review). Moreover, pathogens can hijack or misuse host proteolytic systems. For instance, *S. aureus* (37), *Haemophilus influenzae* (38) and *Pseudomonas aeruginosa* (39) among other common pathogenic bacteria, manipulate the plasminogen-plasmin system thereby degrading laminin and fibronectin and activating MMP zymogens that not only degrade all types of ECM proteins, but also process many biologically active molecules (e.g. cell surface receptors and cytokines) that direct cell behaviour and host defence.

Strategies to evade the host immune response?

Although numerous pathogens undoubtedly exploit ECM components and ECM-associated molecules to adhere to and degrade tissues for efficient host colonization and invasion, it is not always clear whether pathogens interact with these ECM molecules also to evade immune responses. For instance, *B. burgdorferi* can avoid antibody-mediated clearance and this may be partly explained by its specific interaction with decorin (36). The *H. influenzae* surface protein E (PE) binds to plasminogen, which is converted to plasmin, and uses plasmin for complement evasion and innate immune escape (37). Several other pathogenic bacteria take advantage of the host plasminogen system to facilitate their own spread an invasion through tissues (40, 41). Notably, *H. pylori* benefits the complement regulatory property and the plasminogen binding ability of vitronectin to protect itself from innate immune responses (42). Furthermore, Group B *Streptococci* (GBS) and other Gram-positive bacteria secrete hyaluronidases, whose activity allows immune evasion besides tissue invasion. Specifically, their hyaluronidases process pro-inflammatory hyaluronan fragments into disaccharides, which block TLR2/4 signalling triggered by host-derived hyaluronan fragments and pathogenic ligands, including lipopolysaccharide (LPS), thereby evading immune detection (43).

Altered ECM dynamics in infection and its implications

The ECM undergoes significant alterations upon infection that promote or inhibit the establishment of infection and the host response to it. Below is an overview of the changes in ECM synthesis, degradation and post-translational modification during infection and a discussion of their implications in pathogenesis.

ECM synthesis and deposition

Signal transduction pathways activated upon pathogen entry and recognition and by mediators of inflammation and tissue repair during infection all contribute to ECM synthesis and deposition. This involves structural ECM proteins (e.g. collagens, laminins and proteoglycans) as well as non-structural ECM components termed 'matricellular proteins', which are normally absent or scarcely expressed in healthy tissues (e.g. osteopontin, thrombospondins, galectins, tenascins, etc.). For instance, rhinovirus activates TLR3 and TLR7/8 signalling in airway smooth muscle (ASM) cells that leads to increased deposition of fibronectin, perlecan and collagen IV, contributing to airway remodelling and facilitating the migration of ASM cells to the infection site (44). IL-33 signalling induced by *S. aureus* enhances fibronectin and collagen IIIa expression and deposition, accelerating wound repair (45). Systems biology approaches elucidating the ECM interactome network regulated by *Trypanosoma cruzi* and its gp83 ligand, which mediates trypanosome attachment and entry, have shown that activation of gp83 receptors in the cell via ERK1/2 results in upregulation of laminin γ-1 and thrombospondin expression to facilitate trypomastigotes recruitment, enhancing cellular infection (46). In mice, *Citrobacter rodentium* infection induces osteopontin and fibronectin expression through integrin-linked kinase activation, facilitating bacterial colonization of the intestine (47). High expression levels of osteopontin have also been shown to be induced in murine acute and chronic coxsackievirus (CV) B3-myocarditis together with those of MMP-3, TIMP1, urokinase-type plasminogen activator (uPA) and TGF-β1 and, in turn, procollagen-1alpha mRNA expression and fibrosis. Accordingly,

osteopontin-null mice are protected from viral myocarditis and inhibition of osteopontin transcription by vitamin D decreases cardiac fibrosis in wild type animals (48). Viral myocarditis also results in upregulation of tenascin-C before immune cell infiltration occurs and until scar tissue is formed (49). Elevated tenascin-C levels have also been reported in patients with sepsis, parapneumonic infection, tuberculosis and *S.aureus* infection (50-54). Furthermore, the lungs of mice infected with gram-negative bacteria show accumulation of versican, a chondroitin sulfate proteoglycan with multiple cytokine, chemokine, adhesion molecule and growth factor binding domains, that is implicated in the innate immune response (55). Similarly, when primary human lung fibroblasts are treated with the viral mimetic Poly I:C, they deposit a higher-order structured ECM, rich in versican and hyaluronan, to which T-cells avidly adhere and cease migration, an effect reversed by hyaluronidase treatment or versican antibody during matrix formation (56). Importantly, besides expression levels, infection can also alter the spatial distribution of ECM components as it emerges from influenza-infected lungs, which show distinct regions that are enriched with either fibronectin or collagen (57). Thus, specific infectious diseases seem to generate distinct compositional changes of the ECM, inevitably influencing its biophysical structure and presentation of bioactive compounds that impact bacterial colonization and invasion, immune cell response and tissue repair.

ECM degradation

The catabolic machinery that breaks down and remodels the ECM is also altered upon infection, affecting the supportive, barrier and biological functions of the ECM. Tissue degrading enzymes such as MMPs play a crucial role in regulating immune cell recruitment: they cleave the basement membrane ECM; expose cryptic pro-migratory sites of ECM components (e.g. γ2 chain of laminin 5); target non-ECM proteins such as adhesion molecules (e.g. E-cadherin); activate, deactivate or regulate the bioavailability of chemokines (e.g. MCP-1 and IL-8) and cytokines (e.g. IL-1β and TNF-α); and shed cell surface receptors

associated with cell migration (e.g. CD44), modulating inflammation (reviewed in (58-60)). With the exception of neutrophils, tissue degrading enzymes are not stored, but require *de novo* synthesis that is strictly regulated and can be induced by pro-inflammatory cytokines (e.g. TNF-α and IL-1) and also by bacterial products (e.g. LPS and chlamydial heat shock proteins) (61).

Several pathogens, including *Mycobacterium bovis, Mycobacterium tuberculosis*, *S. pyogenes* and *H. pylori,* induce expression and activity of a number of MMPs, including MMP-1, MMP-2, MMP-7, MMP-9 and MMP-13 (62-64). In particular, the extensive production of MMP-2 and MMP-9 during mycobacteria infection is regulated by macrophage- and T-cell-derived cytokines and causes ECM breakdown. This may be necessary for cell recruitment and granuloma formation, both protective immune responses to mycobacteria. However, dysregulation of MMP production at late stages of the infection could contribute to tissue damage and, by compromising tissue integrity, may facilitate bacterial dissemination and persistence of infection (62). Systemic *E. coli* infection, acute Lyme neuroborreliosis and pneumococcal meningitis can all lead to secretion of high amounts of MMP-9. In meningitis, MMP-9 has been suggested to contribute to blood-brain barrier destruction and neuronal injury (65-67). Similarly, gastrin-dependent induction of MMP-7 upon *H. pylori* infection has been implicated in the development of gastric cancer through the release of heparin-binding epidermal growth factor (EGF) (64). *Chlamydia trachomatis*, which causes trachoma and blindness, upregulates MMP-7, MMP-9, MMP-12 and TIMP-1 expression, while it downregulates MMP-10 and SPARC (secreted protein, acidic, cysteine-rich)-like 1, a matricellular protein that regulates decorin production and collagen assembly (68). In this study the expression pattern of these ECM modifying enzymes correlated with the clinical scarring grade and inflammation. Interestingly, a dual function has been shown for MMP-12 in viral myocarditis caused by coxsackievirus type B3. While intracellular MMP-12 causes IFN-α secretion and host protection, extracellular MMP-12 cleaves the IFN-α receptor 2

binding site of IFN-α, preventing an uncontrolled immune response (69). In the same model of viral myocarditis, MMP-9 exerts a protective role by inactivating IFN-β/γ. Indeed, MMP-9-null mice display higher viral load, infiltration of $CD3^+$ cells and tissue damage (70). However, murine MMP-9 can also enhance susceptibility to infection and increase morbidity and mortality. This is the case of *Francisella tularensis* pulmonary infection where MMP-9 generates pro-inflammatory ECM-derived peptides (i.e. Pro-Gly-Pro (PGP) peptide from collagen I), enhancing neutrophil infiltration to lungs (71).

In addition to inducing MMP expression, pathogens can also activate pro-MMPs by secreting their own activating enzymes as in the case of *P. aeruginosa*, which activates pro-MMP-2 using LasB, a thermolysin-like metalloprotease (39). The role of dysregulated tissue degrading enzymes, individually or in combination, is not well understood in every infectious disease. However, it is clear that the MMP/TIMP system changes the ECM composition and biophysics and its presentation of bioactive molecules, generating environmental cues that are detected and processed by immune cells into signalling events that direct their behaviour and response to infection.

ECM post-translational modification

Post-translational modifications (PTMs) add complexity to the ECM. Some are generated by proteolytic cleavage, while others by citrullination of arginine, glycosylation, cross-linking, hydroxylation of prolines, nitrosylation of tyrosines and aspartate isomerization (reviewed in (7)). Given the ability of pathogens to hijack host enzymes or secrete enzymes targeting host molecules, it is tempting to ask whether PTMs in the ECM are altered in infection. Indeed, the *Porphyromonas gingivalis* enzyme peptidylarginine deiminase (PAD) can convert arginine residues to citrulline in mammalian ECM proteins, including fibrinogen, collagen II, fibronectin and tenascin-C (72-76). While arginine is positively charged at neutral pH,

citrulline is uncharged, increasing protein hydrophobicity and, thus, altering protein 3D structure and function. Notably, *P. gingivalis* periodontal infection has been linked to rheumatoid arthritis, an autoimmune disease of the joints characterised by high levels of citrullinated proteins and anticitrullinated ECM protein antibodies (77). *T. cruzi*, the etiologic agent of Chagas disease, which features extensive inflammation and fibrosis of the heart, has been reported to increase the expression of lysyl oxidase (LOX) (78). This enzyme carries out cross-linking of collagen fibers thereby altering matrix stiffness, which has been linked to cancer metastasis (79). By irreversibly altering collagen structure and function, LOX has been proposed to cause dysfunction of cardiomyocytes and, in turn, of the heart in Chagas disease. Similarly, dengue virus infection has been shown to suppress the expression of cartilage associated protein (CRTAP), alongside the protein associated to tight junctions (PATJ). CRTAP associates with the proteoglycan leprecan, which has collagen prolyl 3-hydroxylase activity, and cyclophilin B in the endoplasmic reticulum. This trimeric complex is required for proline 3-hydroxylation of collagen and, thus, collagen assembly. As the levels of CRTAP mRNA negatively correlate with viral replication, the authors of the study have speculated that CRTAP, together with PATJ, restrict dengue infection by influencing cell-cell adhesion (80).

While evidence supporting a link between specific PTMs of ECM proteins and disease such as arthritis and cancer is accumulating, the implications of altered PTMs of ECM components during infection are only beginning to emerge. For example, collagen fiber cross-linking mediated by LOX can significantly alter tissue structure and ECM mechanics. Mammoto *et al*. recently showed that LOX-dependent changes in ECM mechanics control vascular permeability and pulmonary oedema. *In vivo*, mouse lungs treated with LPS, which contribute to pulmonary oedema and acute respiratory distress syndrome in patients with sepsis (81), become much stiffer than non-treated lungs and exhibited enhanced vascular permeability. Increased LOX expression and LOX and LOX1 protein isoform activity control

alveolar architecture and vascular permeability, which are restored by LOX activity inhibition (82).

Macrophages: not just destroyers of the ECM

In bacterial and viral infection, activated macrophages (e.g. M(IFNγ), M(LPS)) have long been implicated in ECM destabilization and destruction through the secretion of tissue degrading enzymes, including MMP-9. Similarly, parasitic infections involve activated macrophages (i.e. M(IL-4)), which release proteases such as MMP-1 and MMP-12 (83). However, macrophages can also produce a number of ECM proteins. Increasing evidence of this and its implications are discussed below.

Fibronectin is the first ECM glycoprotein reported to be produced by human macrophages and IFN-γ–stimulated mouse peritoneal macrophages (84, 85). Moreover, bacterial components induce tenascin-C expression in human monocyte-derived and mouse bone-marrow derived macrophages (53, 86). LPS from *P. gingivalis* induces thrombospondin-1 production in THP-1 cells (87), while LPS from *E. coli* and IFN-γ upregulate galectin-1 and -3, including 5 galectin-3 truncated forms, in primary human macrophages (88). In mice with viral myocarditis, macrophages infiltrating the heart are the main producers of osteopontin (48). Notably, in murine macrophages, LPS causes the formation of chromosomal loops in the osteopontin promoter by bridging NF-kB and AP-1 together, leading to osteopontin transcription (89), which is negatively regulated by GSK3b (90).

Macrophage expression of proteoglycans has also been demonstrated. Treatment of bone marrow-derived and alveolar macrophages with *E. coli* LPS results in rapid induction of versican and hyaluronan synthase 1, and simultaneous inhibition of the major hyaluronan

degrading enzymes (hyaluronidases 1/2) (91). Serglycin, decorin and biglycan are also secreted by LPS-activated mouse peritoneal macrophages (92-94). By using inhibition, chromatin immunoprecipitation and NF-kB reporter gene assays, some of these studies have demonstrated that pathogenic activation of the NF-kB signalling pathway downstream of TLR4 leads to ECM molecule transcription (86, 89, 93-95). Importantly, in the case of tenascin-C, biglycan and decorin, which can activate TLR4, this can promote autocrine loops of inflammation (86, 93, 94). Thus, the infected cellular microenvironment influences TLR function and, in turn, TLR activation affects the microenvironment.

Intriguingly, there is mounting evidence that macrophages can synthesize collagen molecules. In *Drosophila*, phagocytes (hemocytes) produce functional collagen IV, which controls key signalling events in the germline stem cell niche (96). The first reports of collagen synthesis by mammalian macrophages were published in the 1990s. The first showed collagen I synthesis in mouse peritoneal macrophages and the second demonstrated collagen VIII synthesis and secretion in human macrophages that was decreased by IFN-γ and LPS treatment (97, 98). Collagen VIII is a short-chain, nonfibrillar collagen that forms unique hexagonal lattice structures and possesses both structural and signalling properties. Later, human macrophages were shown to secrete collagen VI, which forms beaded filaments with a multidomain structure that interact with ECMs and cell surface receptors to anchor interstitial structures and cells within tissues. Expression of collagen VI was decreased by IFN-γ and LPS, but increased upon IL-4 treatment (99). Recently, the expression of all 28 collagen-encoding mRNAs was quantified in steady-state and LPS-activated primary human macrophages and compared to that of human dermal fibroblasts, an abundant matrix source (95). Steady-state macrophages expressed basal levels (lower than those in dermal fibroblasts) of collagen mRNAs with the exception of collagen III, X, XI, XVI, XX and XXVI. However, LPS specifically increased the expression of fibril-associated collagens with interrupted triple helices (FACITs; collagen VII, XII, XV, XIX and XXI), the

collagenase-resistant collagen V, the collagen-containing von Willebrand factor collagen XXVIII and collagen I, IV, XVIII, XXV and XXVII. Collagen II, VIII, IX, XIII, XIV, XXIII and XXIV expression was not increased. LPS also downregulated the expression of collagen VI, confirming previous studies (99), and collagen XIII and XVII (95). By secreting collagens, depending upon their mode of activation, macrophages may contribute to the ECM and therefore tissue stabilization and repair and to cell-cell and cell-matrix interactions (e.g. *in vitro*, monocytes adhere strongly to collagen VI (99)). Furthermore, macrophages may bind to their secreted collagen molecules as they express several receptors known to interact with collagen (e.g. integrins and proteoglycans). However, collagen secretion by macrophages and its potential role in macrophage adhesion and immune response *in vivo* remain to be clarified.

The ECM: an integral part of the innate immune response to infection?

Antimicrobial activity of the ECM

The innate immune system employs endogenous peptides like alpha-defensin and LL-37, which bind to heparin and dermatan sulphate glycosaminoglycans and have antimicrobial properties. During the inflammatory response to infection, tissue degrading enzymes generate bioactive ECM fragments. A number of studies showed that heparin-binding peptides derived from laminin isoforms, vitronectin, thrombospondin and fibronectin exert antimicrobial activities against gram-positive and gram-negative bacteria, and the fungus *Candida albicans* (100-102).

Certain ECM proteins are also found in biological fluids. One such protein is tenascin-C, which has been found in human breast milk (2.2-671 μg/ml) where it acts as an innate broad-spectrum HIV-1-neutralizing protein (103). Tenascin-C directly captures HIV-1 virions

by binding to the HIV-1 Envelope gp120 protein at a CD4-inducible epitope that overlaps the chemokine coreceptor binding site. Accordingly, tenascin-C depletion abolishes the HIV-1-neutralizing activity of milk (103).

Direct antimicrobial activity has also been reported for the ECM-associated protein MMP-12, which is abundantly expressed in mature tissue macrophages and mobilized to macrophage phagolysosomes after the ingestion of bacteria. Inside phagolysosomes, MMP-12 adheres to bacterial cell walls and disrupts cellular membranes, killing the bacteria. Notably, the C-terminal domain of MMP-12, but not its catalytic domain, contains a four amino acid sequence on an exposed beta loop of the protein that is unique in nature and confers antimicrobial activity (104). Together, these data may help the search for safe, endogenous antimicrobial molecules from complex biological mixtures. Furthermore, binding epitopes could serve as templates for *de novo* synthesis of novel antimicrobial molecules.

ECM-mediated recognition of microbial pathogens, macrophage activation and phagocytosis

The innate immune system employs highly conserved receptors, namely pattern-recognition receptors (PRRs), to recognize conserved motifs in microbial pathogens, called pathogen-associated molecular patterns (PAMPs). Mindin, a member of the mindin-F-spondin family of secreted ECM proteins, has emerged as a unique pattern-recognition molecule in the ECM for microbial pathogens and has been proposed to function as an integral part of the innate immune response (105). He *et al*., showed that genetic ablation of mindin confers resistance to LPS-induced shock and systemic *S. typhimurium* and *S. pneumoniae* infections *in vivo*. Moreover, mindin-null mice feature impaired bacterial clearance in lungs infected with gram-positive group B streptococcus or *H. influenza. In vitro*, macrophages and mast cells lacking mindin display impaired TNF-α and IL-6 production and defective phagocytosis. By using recombinant mindin, the authors showed that this ECM protein recognizes carbohydrate moieties of gram-positive and gram-negative bacterial components and, by binding to them,

it agglutinates bacteria. As glucose inhibited not only mind binding to pathogens but also macrophage activation by LPS, the authors concluded that mindin-mediated carbohydrate recognition of microbial pathogens is a secondary stimulation necessary for the activation of macrophages and mast cells (105) (Fig. 1). Notably, mindin is not a universal, but a rather specific pattern-recognition molecule as it only recognizes and opsonizes certain bacteria. Following this, the innate immune function of mindin has been extended to include promoting influenza virus clearance from the nasal cavity by allowing efficient macrophage activation (106). Furthermore, the proteoglycan lumican, whose core protein contains tandem repeats of leucine rich motifs similarly to PRRs, interacts with CD14 on the surface of macrophages and neutrophils, promoting CD14-TLR4-mediated responses to LPS (Fig. 1). Thus, lumican-null mice are hypo responsive to LPS-induced septic shock (107). In a *P. aeruginosa* model of lung infection, mortality of lumican deficient mice is increased as animals fail to clear bacteria from tissues. This study showed that CD14-mediated phagocytosis of *E. coli* and *P. aeruginosa* bacteria by macrophages is impaired in the absence of lumican and identified Tyr-20 as a vital residue for CD14 binding and phagocytosis (108). Finally, infection of the cornea with *P. aeruginosa* readily increases lumican expression before inflammatory cell infiltration and lumican-null mice display poor resolution of bacterial keratitis and sustained production of pro-inflammatory cytokines (109). Another ECM protein that senses a number of microbial pathogens is galectin-3, which binds to carbohydrate structures on glycoproteins and glycolipids (e.g. N-acetyl-D-lactosamine and LPS) from (myco)bacteria, protozoan parasites and yeast (110). Although the *in vivo* function of galectin-3 during infection has not been fully investigated, upon microorganism recognition, it contributes to macrophage-mediated phagocytosis, at least *in vitro* (111) (Fig. 1). Whether these ECM components exert the same essential immune functions in humans represents an outstanding, important question.

Leukocyte population balance in infection: emerging roles for ECM components

Severe infection demands large numbers of leukocytes that is compensated by the emergency myelopoiesis response, which is initiated by activated PRRs and cytokines, including IL-6, GM-CSF and G-CSF. This protects the host from systemic infection by quickly generating the required leukocyte population. Kanayama *et al*. have recently found that osteopontin skewed the balance of myeloid and lymphoid cell populations during systemic infection with *C. albicans* (112). Specifically, the authors showed that osteopontin limited the supply of neutrophils and $Ly6C^+$ monocytes-macrophages by enhancing the apoptosis of common myeloid and granulocyte-macrophage progenitors through a downregulation of the expression of the apoptosis inhibitor survivin. This resulted in greater fungal load in kidneys and significantly higher mortality of wild type mice with systemic fungal infection compared to osteopontin deficient littermates. The detrimental effect of osteopontin was observed early, 24 hours post infection (112). However, it is unclear if this effect is maintained, exacerbated or reversed at later stages of infection.

Dendritic cells (DCs) are the main antigen-presenting cells and, upon capturing microbial pathogens, mature and migrate to lymphoid tissues where they activate naïve T cells. Distinct classes of microbes elicit lineage-specific responses from the effector T cell repertoire. Helper T1 (Th1) cells are involved in infection by intracellular bacteria and viruses, Th2 cells in parasitic infection, and Th17 in infection by extracellular pathogens and facultative and obligate intracellular bacteria and fungi. Regulatory T cells (Tregs) hold the inflammatory response in check. Specific ECM proteins have been shown to contribute to the T cell polarizing function of DCs without affecting DC development. For instance, generation of Th17 cells by *E. coli* LPS or *M. tuberculosis* stimulated bone marrow-derived DCs from tenascin-C-null mice is significantly impaired (113). Furthermore, the expression of galectin-3 in DCs controls the magnitude of T cell priming *in vitro* and *in vivo* during helminthic infection with *Schistosoma mansoni*. Galectin-3 deficient mice have significantly

less T cells in their spleen and higher cellular and humoral Th1 responses (114). Although this study shows that galectin-3 expression by DCs modulates the proliferation and cytokine release by T cells, it does not explain the mechanism responsible for the biased Th1 response. Efficient T cell priming by DCs has also been shown to depend on mindin (115). When DCs from mindin-null mice are activated with bacterial components, including LPS from *Salmonella typhosa* or *E. coli* and lipoteichoic acid from *S. aureus*, $CD4^+$ T cell priming is 60-70% lower than that of wild type mice. Investigation of this demonstrated that DCs interact with mindin via integrins $\alpha_4\beta_1$ and $\alpha_5\beta_1$, leading to upregulated expression of the Rho GTPases Rac1/2, which are known to regulate DC priming of T cells (115). As DCs link innate to adaptive immunity and unbalanced effector T cell populations leads to pathological inflammation, understanding how the ECM can regulate T cell responses is crucial.

Transcriptional and post-transcriptional roles for ECM molecules in host defense signalling pathways

Pathogen recognition via PRRs initiates inflammatory signalling pathways which are tightly regulated to allow microbial clearance with minimal damage to the host. Recent research implicates a role for ECM and ECM-associated proteins in regulating inflammatory networks at the transcriptional and post-transcriptional level during the immune response to infection.

An elegant study by Marchant *el al*. found a transcriptional role for MMP-12 (macrophage elastase) in immunity against viral infection (69). During coxackievirus type B3 and respiratory syncytial virus infections, MMP-12-null mice display increased viral load, mortality and lower levels of IFN-α, which is essential for viral immunity. Mechanistically, secreted MMP-12 is taken up by virus-infected cells and traffics to the nucleus, where it binds to the *NFKBIA* promoter, driving its transcription, which is essential for optimal IFN-α secretion and host protection. Additionally, MMP-12 regulates specific substrates by two distinct

mechanisms: 1) through DNA binding of gene exons (e.g. exons encoding PSME3, the immunoproteasome cap protein, and secreted protein acidic and rich in cysteine (SPARC)-like protein 1, which decreases their mRNA and protein levels in MMP-12-null mice); and 2) extracellularly, through substrate protein cleavage (e.g. INF-α receptor 2 binding site). Thus, MMP-12 clears systemic INF-α and, accordingly, selective inhibition of extracellular MMP-12 in infected wild type mice elevates systemic INF-α levels and reduces viral replication (69).

At the post-transcriptional level, a role for decorin and tenascin-C has been found in regulating miRNAs in LPS-induced sepsis (53, 93, 116). Merline *et al*. detected increased decorin levels in septic patients and mice with LPS-induced sepsis, and elevated IL-10 amounts in decorin-null mice. They demonstrated that, in the presence of LPS, decorin reduces the levels of the anti-inflammatory cytokine IL-10 via two mechanisms. Firstly, it activates ERK and p38 MAP kinases downstream of TLR2 and TLR4 thereby inducing the expression of the pro-inflammatory modulator PDCD4 (programmed cell death 4), which translationally represses IL-10. Secondly, decorin inhibits TGF-β1 signaling, leading to lower levels of miR-21, which represses PDCD4 expression and thus decreases IL-10 levels (93). Soon after, tenascin-C was shown to orchestrate the secretion of specific cytokine subsets in response to LPS. Specifically, tenascin-C regulates the biosynthesis of the LPS-responsive miRNA miR-155, allowing optimal TNF-α production in macrophages and effective immune response to LPS *in vivo* (53). Thus, specific ECM and ECM-associated proteins possess previously unknown transcriptional and post-transcriptional regulatory activities, which fine-tune the innate immune response to infection.

The ECM in infectious granulomas

Tuberculosis, syphilis, toxoplasmosis, infectious mononucleosis and measles are a few examples of infectious diseases characterized by the formation of granulomas. Traditionally considered host-protective structures, infectious granulomas are compact, organised immune cell clusters that are generated in response to specific pathogens. Granulomas contain large numbers of mature macrophages, which can fuse into multinucleated giant cells or differentiate into foam cells. Neutrophils, dendritic cells, B, T and natural killer cells, and fibroblasts are also found in granulomas, which are surrounded by epithelial cells. Granulomas also contain ECM proteins, whose expression and function in infectious granulomatous disease pathogenesis has only recently been investigated.

In humans, osteopontin has been detected in granulomas of diverse etiology (117) and, in those caused by *Paracoccidioides brasiliensis*, it localizes in ECM, macrophages and multinucleated giant cells at the center of lesions in the early phase of infection (118). In tuberculosis granulomas, osteopontin is markedly expressed within lymphocytes, macrophages, epithelioid cells and multinucleated giant cells, but not in the central necrotic core (54). These granulomas also stain strongly for tenascin-C in the surrounding fibrotic rings and weakly inside, in the ECM. A diffuse ECM-associated staining of galectin-9 is detected in the granuloma and in epithelioid and multinucleated giant cells. Notably, osteopontin, tenascin-C and galectin-9 are absent in non-infectious Crohn disease granulomas (54). Analysis of human lung biopsies from patients with atypical mycobacteriosis and tuberculosis revealed expression of tenascin-C and precursor proteins of collagens I and III around granulomas. Precursor proteins of collagen I were also found within granulomas that colocalized with myofibroblasts (119). In mice infected with *P. brasiliensis*, Gonzalez *et al*. detected increased expression of laminin, fibronectin, fibrinogen, collagen I, collagen III, elastic fibers and proteoglycans during granuloma formation (120). Analysis of their arrangement showed that they were mostly surrounding the granuloma, but

sporadically inside it. Initially arranged in a disorganised manner, ECM fibres later acquired a compact, concentric arrangement, which originated from an anchorage point that may contribute to tissue integrity and enhance distribution of growth factors and cytokines (120). Notably, during chronic mycobacterial infection, the fibrinolytic system has been shown to limit progressive fibrosis with plasminogen regulating the turnover of ECM proteins within the granuloma (121).

Recent studies on ECM turnover in tuberculosis granuloma has helped rewrite tuberculosis immunopathology. Traditionally considered a host-protective structure that 'quarantines' the infecting mycobacteria, tuberculosis granuloma has been implicated in the expansion and dissemination of infection. Specifically, caseous necrosis leading to ECM destruction and bacterial dissemination was thought to be the cornerstone of tuberculosis pathogenesis. Conversely, collagen destruction has now been proposed to initiate caseous necrosis. Elkington *et al*. have first shown that MMP-1-expressing mice develop collagen destruction within granulomas upon infection with *M. tuberculosis* in the absence of caseous necrosis (122). They then demonstrated that proteolytic collagen destruction of the lung ECM is the initial pathological event that reduces the survival of *M. tuberculosis*-infected cells, resulting into caseous necrosis and cavitation, thus diverting the immune response in favour of the pathogen. Conversely, intact collagen fibrils increase survival of infected cells (123, 124). In line with this, Parasa *et al*. reported upregulation of MMP1, 3, 9 and 12 in a human lung tissue model and in biopsies from patients with non-cavitary tuberculosis. Global MMP inhibition via marimastat reduced granuloma formation and bacterial load (125).

Future work should take into account the hypoxic conditions in infectious granulomas and include unbiased global analyses of ECM and ECM-associated molecules in granulomas at various disease stages and gain- and loss-of-function experiments in 3D systems and/or

genetically modified animals to understand the role of the ECM in granuloma formation and function.

Concluding remarks and future perspectives

The ECM and the innate immune response to infection are inextricably linked. Largely ignored or overlooked, the diverse yet specific functions of the ECM in infection influence the establishment and dissemination of microbial pathogens in host tissues as well as the outcome of the immune response to the infection (Fig. 2). In addition to the studies discussed here, ECM-immunological research, which is expanding into the areas of prion disease (126), virus transmission (127), exosomes in viral pathogenesis (128) and vaccine development (129), shows the breadth and complexity of ECM activity and regulation in infection.

The ECM undergoes significant changes upon microbial invasion, magnifying the complexity of the cellular microenvironment at sites of infection. This presents challenges in setting up appropriate model systems and identifying the ECM and immunological pathways that are directly responsible for the outcome of individual infectious diseases. Unbiased omics, systems biology and genome editing approaches are promising resources for defining these pathways, designing mechanistic studies, and, in the longer-term, elucidating them in the context of the microbiota.

As antimicrobial resistance is of global concern and tackling it is becoming increasingly challenging, investigating the now evident role of the ECM in infection may reveal novel therapeutic strategies or improve existing ones. It may also inform biomaterials and tissue engineering in their effort to prolong the lifespan and integrity of medical devices that are compromised by infection.

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Disclosures

None to declare.

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Figure legends

Figure 1. Mindin, lumican and galectin-3: three extracellular sentinels. Mindin, lumican and galectin-3 recognize and bind to sugar moieties found in the cell wall of several types of bacteria. All of them promote phagocytosis of bacteria by macrophages. Mindin binds to bacteria, causes their opsonisation and agglutination, and facilitates their phagocytosis by macrophages. Mindin also induces the synthesis of proinflammatory cytokines by these cells. Lumican instead interacts with CD14 on the surface of macrophages, promoting CD14-TLR4-mediated responses to LPS and CD14-mediated phagocytosis.

Figure 2. Multiple functions of the ECM in the immune response to infection. Recognition of pathogen-associated molecular patterns by macrophages through PRRs and by ECM components is shown. Pathogen binding to ECM molecules such as fibronectin helps host colonization. Degradation of the ECM via microbial tissue degrading enzymes or host MMPs activated by pathogens facilitates host invasion. To establish the infection, pathogens can also hijack host proteolytic systems such as the plasminogen-plasmin system and evade innate immune responses by binding to ECM components such as vitronectin.

Fibroblasts in the interstitial ECM produce and secrete ECM proteins, MMPs and higher levels of LOX, which crosslinks collagen fibers, increasing ECM stiffness. Pathogen-mediated activation of macrophages triggers inflammatory signalling pathways such as the NF-kB pathway, which culminates in the synthesis of cytokines, MMPs and miRNAs. Activated macrophages synthesize also ECM components such as decorin and tenascin-C, which regulate the biosynthesis of miR-21 and miR-155 and generate positive feedback loops that propagate inflammation.

Tables

Table 1. Examples of specific host ECM-pathogen interactions that facilitate microbial adhesion to tissues and pathogenesis (***** indicates that animal models of infection were used in the study)

ECM molecule	Pathogen	Adhesin	Effect of interaction	Disease	Reference
Fibronectin	Enterohemorrhagic *Escherichia coli* O157:H7	Lpf fimbriae (LpfA1 major subunit)	Colonization of GI tract	Acute diarrhea; bloody diarrhea; hemolytic uremic syndrome	(130)
	Streptococcus pyogenes (group A streptococci, GAS)	Protein F1 (functional upstream domain)	Fibronectin links F1 to integrin receptors, helping bacterial uptake	Tonsillopharyngitis; necrotizing fasciitis; myositis; streptococcal toxic shock syndrome	(131-133)
		Protein F2 (C-terminal domains)	Host cell adhesion and internalization		(33)
		SfbII (C-terminal domain)	Host cell adhesion and internalization		(134)
		Fba	Host cell adhesion and internalization		(31) *****
	Staphylococcus aureus	MntC	Mucosal colonization	Nosocomial infections,septicaemia, osteomyelitis, endocarditis, etc.	(135)
	Salmonella typhimurium	MisL (N-terminal non-conserved region)	Intestinal colonization	Gastroenteritis	(136) *****
		ShdA	Binding to fibronectin 13FnIII repeat module and intestinal colonization		(137)
Plasma fibronectin	*Borrelia burgdorferi*	BBK32	Fibronectin polymerization and	Lyme disease	(35)

			colonization of vascular surfaces		
Fibronectin N-terminal proteolytic fragments (30 and 70 kDa)	*Streptococcus pyogenes*	Protein F2 (C-terminal domains)	Eukaryotic cell adhesion and internalization	Tonsillopharyngitis; necrotizing fasciitis; myositis; streptococcal toxic shock syndrome	(33) (130)
Fibronectin N-terminal proteolytic fragments (30, 45 and 70 kDa)	Enterohemorrhagic *Escherichia coli* O157:H7	Lpf fimbriae (LpfA1 major subunit)	Colonization of GI tract	Acute diarrhea; bloody diarrhea; hemolytic uremic syndrome	(130)
Laminin	Enterohemorrhagic *Escherichia coli* O157:H7	Lpf fimbriae (LpfA1 major subunit)	Colonization of GI tract	Acute diarrhea; bloody diarrhea; hemolytic uremic syndrome	(135)
		MntC	Mucosal colonization		
	Staphylococcus aureus			Nosocomial infections, septicaemia, osteomyelitis, endocarditis, etc.	(138)
		FimB, gtf and pilB	Adhesion and invasion of endothelial cells		
	Streptococcus gallolyticus (*gallolyticus* endocarditis isolates)			Infective endocarditis	(139)
		ErpX	Long-term host tissue colonization		
Collagen I	*Borrelia burgdorferi*				(138)
		FimB, gtf and pilB	Adhesion and invasion of endothelial cells	Lyme disease	
	Streptococcus gallolyticus (*gallolyticus* endocarditis isolates)			Infective endocarditis	(137)
		ShdA	Intestinal colonization and persistence		
	Salmonella typhimurium			Gastroenteritis	(140) *
		Pilus subunits EmpA and EmpB	Adherence to host tissue and biofilm formation		
Collagen II	*Enterococcus faecium*			UTIs, bacteremia, and infective endocarditis	(138)
		FimB, gtf and pilB	Adhesion and invasion of endothelial cells		
	Streptococcus gallolyticus (*gallolyticus*				

Collagen IV	endocarditis isolates)			Infective endocarditis	(130)
	Enterohemorrhagic *Escherichia coli* O157:H7	Lpf fimbriae (LpfA1 major subunit)	Colonization of GI tract		(135)
		MntC	Mucosal colonization	Acute diarrhea; bloody diarrhea; hemolytic uremic syndrome	
	Staphylococcus aureus				(138)
	Streptococcus gallolyticus (*gallolyticus* endocarditis isolates)	FimB, gtf and pilB	Adhesion and invasion of endothelial cells	Nosocomial infections,septicaemia, osteomyelitis, endocarditis, etc.	(136) *
	Salmonella typhimurium	MisL (N-terminal non-conserved region)	Intestinal colonization	Infective endocarditis	
Collagen V					(141)
	Enterococcus faecium	EcbA	Host tissue adhesion and biofilm formation	Gastroenteritis	(142) *
Collagen VI					
	Legionella pneumophila	Mip	Adhesion to lung tissue and bacterial dissemination	UTIs, bacteremia, and infective endocarditis	
Tenascin-C					(138)
	Streptococcus gallolyticus (*gallolyticus* endocarditis isolates)	FimB, gtf and pilB	Adhesion and invasion of endothelial cells	Legionellosis	
Vitronectin					(138)
	Streptococcus gallolyticus (*gallolyticus* endocarditis isolates)	FimB, gtf and pilB	Adhesion and invasion of endothelial cells	Infective endocarditis	(143) *
	Yersinia enterocolitica	YadA	Adhesion to host cells and tissue; improved bacterial survival	Infective endocarditis	
Thrombospondin					(46)
	Trypanosoma cruzi	TcCRT	Enhancement of cellular infection	Enteric and systemic diseases	(144) *
Decorin		DbpA	Specific localization to		

	Borrelia burgdorferi	and DbpB	decorin-rich niches in the tunica adventitia and myocardial connective tissue; persistence of infection	Chagas disease Lyme disease	(141)
Nidogen 1 and 2					
	Enterococcus faecium	SgrA	Host tissue adhesion and biofilm formation		(145, 146)
Soluble and immobilized fibrinogen (alpha- and beta-chains)	*Staphylococcus aureus*	ClfA, ClfB	Colonization of biomaterial implants; bacterial spread	UTIs, bacteremia, and infective endocarditis Nosocomial infections,septicaemia, osteomyelitis, endocarditis, etc.	

GI: gastrointestinal; UTI: urinary tract infection; LpfAI: long polar fimbrae subunit 1; SfbII: fibronectin surface binding protein II; Fba: fibronectin binding protein; MntC: manganese transport protein C; MisL: autotransport protein MisL; FimB: type I fimbriae regulatory protein FimB; gtf: glucosyltransferase; pilB: type 4 fimbrial assembly protein; EcbA: E. faecium collagen binding protein A; MIP: macrophage infectivity potentiator; YadA: adhesin YadA; TcCRT: Trypanosoma cruzi calreticulin; DdpA/ DdpB: decorin binding protein A and B; SgrA: serine-glutamate repeat containing protein A; ClfA/ClfB: clumping factor A and B.

Table 2. Examples of specific ECM-pathogen interactions that facilitate host invasion through direct degradation of ECM components (***** and # indicate that animal models of infection and *ex vivo* mammalian tissue degradation models, respectively, were used in the study)

ECM molecule	Pathogen	Microbial enzyme	Effect of ECM cleavage	Disease	Reference
Laminin	*Psedomonas aeruginosa*	Elastase; alkaline protease	Tissue invasion and necrosis	Necrotizing pneumonia, septic shock, UTI, skin and soft tissue infections	(147) #
	Clostridium difficile	Cwp84	Tissue integrity loss; facilitation of toxin diffusion	Pseudomembranous colitis and nosocomial diarrhea	(148)
Collagen I	*Porphyromonas gingivalis*	Gingipains in *P. gingivalis* supernatant	Tissue degradation	Periodontal disease	(149)
	Vibrio parahaemolyticus	Metalloprotease VppC	Tissue damage	Acute gastroenteritis	(150)
Collagen I, II, III, IV, V and VI	*Clostridium histolyticum*	Class I and II collagenases (ColG, ColH)	Necrotic tissue degradation; promote keratinocyte migration	Gas gangrene, infective endocarditis	(151, 152)
Collagen IV	*Streptococcus gordonii*	Serine protease	Basement membrane breakdown	Infective endocarditis	(153)
Fibronectin	*Clostridium difficile*	Cwp84	Tissue integrity loss; facilitation of toxin diffusion	Pseudomembranous colitis and nosocomial diarrhea	(148)
	Porphyromonas gingivalis	Gingipains in *P. gingivalis* supernatant; HRgpA and RgpB gingipains	Cleavage and inactivation of cell-binding region of fibronectin; gingival fibroblast detachment and death; tissue destruction;	Periodontal disease	(149, 154) #
Vitronectin	*Clostridium difficile*	Cwp84	Tissue integrity loss; facilitation of toxin diffusion	Pseudomembranous colitis and nosocomial diarrhea	(148)
Tenascin-C (large	*Porphyromonas gingivalis*	HRgpA, RgpB and Kgp gingipains	Enhanced anti-adhesive activity of	Periodontal disease	(154) #

isoforms)			tenascin-C; gingival fibroblasts detachment, apoptosis and tissue destruction		

Cwp84: putative cell surface-associated cysteine protease; HRgpA and RgpB: arginine-gingipains; Kgp: lysine-gingipains.