# The Extracellular Matrix and the Immune System: a mutually dependent relationship

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**Print Summary**

**Background:** The extracellular matrix (ECM) forms a dynamic structure around cells that is essential for the supply of environmental factors, mechanical support and protection of tissues. It includes components such as fibrillar proteins, glycosaminoglycans (GAGs), proteoglycans, and mucus. The molecular, physical, and mechanical properties of the ECM regulate immune cell mobility, survival, and function. In turn, the immune system maintains and regulates healthy matrix and restores matrix integrity following injury. A dysregulated ECM–immune system partnership contributes to most diseases. Exploring the complex interconnectivity between ECM biology and immune cells has the potential to help treat disease and maintain healthy aging.

**Advances:** Immune cells are perpetually in contact with the ECM, and yet the potential consequences of these interactions often remain unexplored. One function of the ECM is to guide immune cell movement and positioning. T cells, for example, move through sites containing thin ECM fibers in preference to more densely cross-linked collagen matrices, whereas heparan sulphate proteoglycans within the vasculature and tissue parenchyma bind and present chemokines to form gradients that direct cell movement. During inflammation, injury, infection, or even aging, ECM components can be released to act as “danger signals”. Conversely, the breakdown of the ECM by matrix-degrading enzymes can generate immunoregulatory fragments. Critically, because cytokines are often bound to GAGs, ECM changes can regulate cytokine availability or activity.

During aging and fibrotic diseases, changes to the fibrillar collagen network result in pathological tissue stiffness and loss of mechanical compliance. Additionally, increases in the GAG hyaluronan contribute to altered ECM mechanical properties that accompany age and disease. There is increasing evidence that immune cell function is regulated by mechanosensing

28 receptors such as Piezo1 and these ECM changes have major impacts on immune function. Furthermore, a decline in the activity of mechanosensing transcriptional activators YAP and TAZ during physiological aging results in failure to downregulate inflammation. Thus, ECM composition actively regulates immune processes, but immune signals will themselves regulate
32 ECM composition, reflecting an essential bidirectional dialogue.

One prominent way the immune system regulates the ECM is via TGF-β, which promotes myofibroblast differentiation and collagen production and inhibits matrix-degrading metalloproteinases. Furthermore, type 2 cytokines, particularly IL-13, have emerged as modulators
36 of ECM quantity and quality, which includes regulating the mucosal barrier. Moreover, direct biophysical interaction with chemokines or cytokines can alter ECM structure and/or function. For instance, CXCL4 (PF4) functions by binding to GAGs rather than directly binding to chemokine receptors. This can lead to remodeling of the cell surface ECM and signaling through
40 proteoglycans.

Immune cells control ECM not only through the production of cytokines and chemokines but by direct synthesis of ECM components and the enzymes that break them down. Enzymatic remodeling of the rigid basement membrane by tissue-infiltrating myeloid cells, for example, can
44 provide routes for lymphocytes to follow. Macrophages, which are pivotal in ECM turnover via receptor mediated uptake of and degradation of collagen, also produce collagens that may provide templates for tissue remodeling. Remarkably, neutrophils can pull and carry pre-existing matrix from nearby sites to wound beds early in the tissue repair process to re-establish new ECM
48 scaffolds.

**Outlook:** The ECM, long considered an inert scaffold, can now be seen as a highly dynamic partner to the immune system. In this review, we aim to highlight the absolute interdependence of these systems with consequences for therapy. For example, immune cell–based therapies may fail if they are placed in diseased matrix that itself drives pathology. Ultimately, to answer the most pressing questions in tissue health, it will be critical that immunologists and matrix biologist work together.

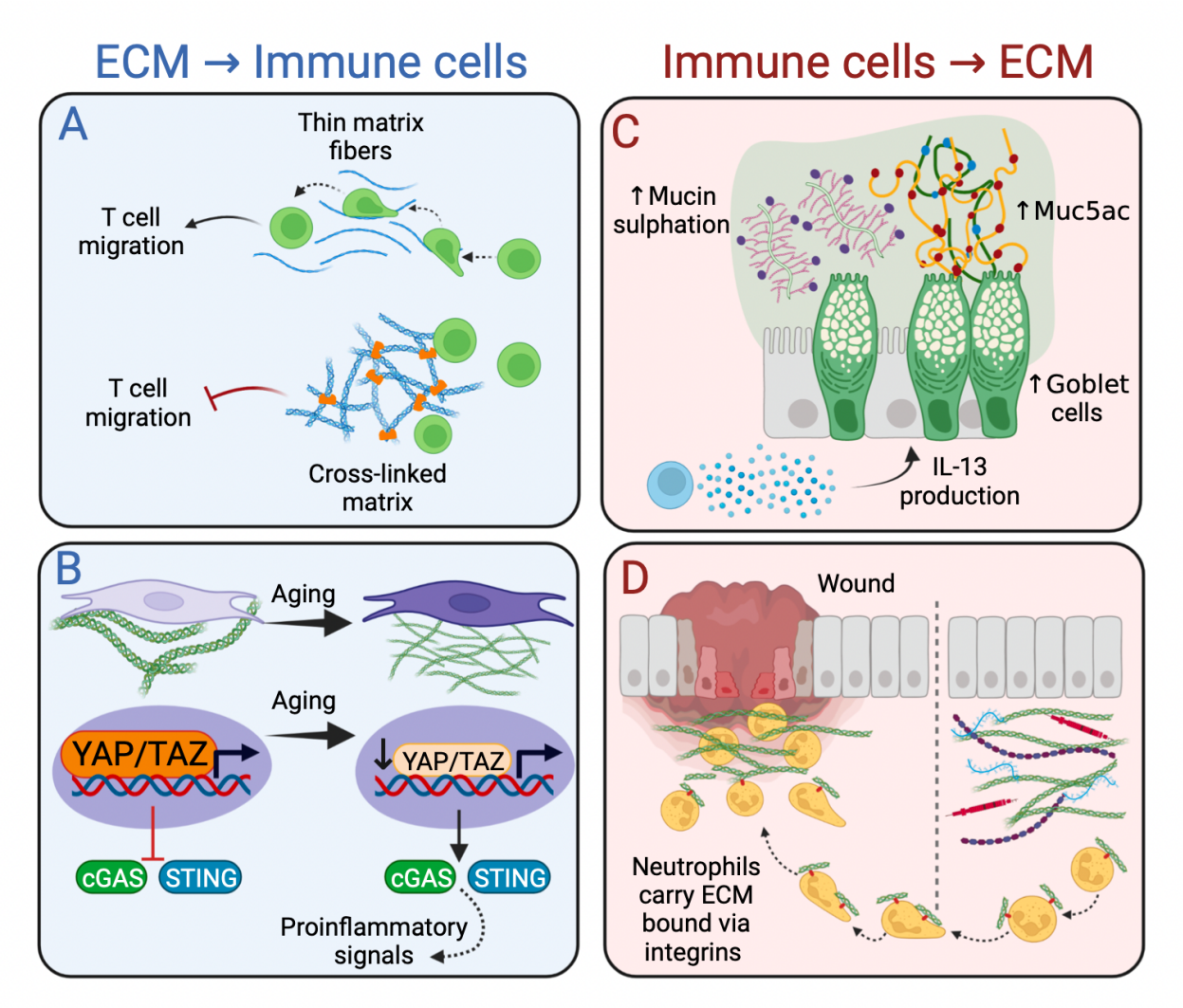

**Figure caption:** Interconnectivity between the ECM and immune system. (A) Physical and molecular properties of the ECM control immune cell positioning and migration within a tissue during pathology and (B) aging alters not only the mechanical properties of ECM, which are sensed by the immune system, but also reduces mechano-sensors that downregulate proinflammatory pathways. (C) The immunomodulatory cytokine IL-13 can remodel the mucus barrier, whereas (D) immune cells themselves can carry matrix across tissues to help build an ECM-scaffold for repair.

**Main Article**

**Abstract:** For decades, immunologists have studied the role of circulating immune cells in host protection, with a more recent appreciation of immune cells resident within the tissue microenvironment and the intercommunication between nonhematopoietic cells and immune cells. However, the extracellular matrix (ECM), which comprises at least a third of tissue structures, remains relatively underexplored in immunology. Similarly, matrix biologists often overlook regulation of complex structural matrices by the immune system. We are only beginning to understand the scale at which ECM structures determine immune cell localization and function. Additionally, we need to better understand how immune cells dictate ECM complexity. This review aims to highlight the potential for biological discovery at the interface of immunology and matrix biology.

The molecular, physical, and mechanical properties of the extracellular matrix (ECM) regulate immune cell mobility, survival, and function. In turn, the immune system is required to maintain and regulate healthy matrix and restore matrix integrity following injury. Thus, the ECM and the immune system work together to preserve tissue health and if this partnership is compromised, disease can develop. However, matrix biology and immunology are typically studied independently. Progress in understanding and treating disease requires that we embrace the complexity, not only of different immune cell–cell interactions, but also their interactions with the matrix that surrounds them, recognizing that the matrix is as complex as the cellular systems it regulates.

The ECM accounts for over one third of our body mass and its dysregulation is the direct or indirect cause of most major chronic diseases. A prime example of this is fibrosis, which accounts for 45% of all deaths (*1*) and can be defined as dysregulated matrix that leads to formation of dysfunctional scar tissue and end stage organ failure. To understand the immunological basis for these diseases, we therefore need an understanding of the interconnectivity between the ECM and immune function. Indeed, immune cell therapies may fail because the cells have been transferred into a diseased matrix that recapitulates a pathogenic signaling processes (*2*). Consequently, a knowledge of matrix biology is essential to aid immunological discovery and unravel mechanisms driving complex diseases. In this review, we highlight the intimate and essential connection between the ECM and immune cells and identify emerging areas of research that are ripe for discovery.

## What is the extracellular matrix?

The ECM forms a structure around cells that is essential for the supply of nutrients and local and systemic factors as well as the removal of waste. It also provides different levels of mechanical

support, characteristic of different tissues. ECM is produced and assembled by the cells living within it and contains components that can be either long-lived or rapidly turned over, making the ECM dynamic and sensitive to local and systemic changes. Diverse ECM functions are reflected by varied composition of a hierarchy of protein and glycan structures. These include fibrillar molecules such as collagen (which give tissue strength and resilience), glycosaminoglycans (GAGs) and proteoglycans (which form hydrated gels that act as a cushion within and around tissues), and mucus (which protects our barrier surfaces). Additionally, a large number of matrix-associated molecules are involved in holding ECM structures together and/or connecting them to the cell surface. Together, ECM components not only provide dynamic tissue organization and integrity, but are signaling molecules in their own right, participating and driving many biological functions. Although many ECM macromolecules are common across tissues, the complex structures they form along with unique modifications are highly tissue-specific (Fig. 1). The biochemistry of ECM molecules including collagen (*3*), sulphated GAGs (*4*–*6*), non-sulphated GAGs (*6*–*8*), mucins (*9*), and the ECM more broadly (*10*, *11*) have been extensively reviewed previously. From an immunological standpoint, matrices also contain a varied spectrum of other secreted proteins including cytokines, chemokines, and growth factors, which can be active, latent, or concealed and are an implicit part of immune cell regulation. It is also notable that cells are often intimately connected to the unique matrix surrounding them and every cell to varying degrees is coated in a glycocalyx, a complex network of sugar-rich molecules either free or bound to protein or lipid (*12*).

## How does the ECM regulate immune cell function?

Immune cells are in contact with the ECM from their early embryonic or bone marrow origins, through to effector functions in the tissues where they transit, or reside, and yet the potential consequences of these interactions often remain unexplored. For example, given the complex structure and function of the ECM, is it easier for a cell to move through a loose hydrated matrix, or to use molecular handles (e.g., integrins) to latch onto and pull through a thicker matrix? The best understood of these processes for immunologists is the process of leukocyte extravasation during an inflammatory event (*13*). However, even here immunologists rarely consider the challenges required for cells to cross through the different ECM environments involved, which includes the endothelial glycocalyx and the basement membrane, as well as interstitial matrix lying beneath endothelial cells that line the blood vessel wall. Furthermore, once immune cells have penetrated the endothelial lining and entered the tissues, the ECM continues to direct immune cell migration. For both migrating and tissue resident cells, the ECM helps determine not only their ultimate location but also regulates their survival and function.

### The endothelial glycocalyx shield

The most studied ECM barrier in the context of leukocyte trafficking and positioning is the basement membrane. This complex network of collagen (type IV & VIII) and laminin—bridged by the glycoprotein nidogen and proteoglycans (e.g., perlecan) (Fig. 1)—lies underneath the vasculature and surrounds most tissues (*14*). However, the first ECM structure encountered by trafficking immune cells is the endothelial glycocalyx lining the vascular endothelium (Fig. 2) (*15*, *16*). The glycocalyx can extend from 200 nm up to 2 μm into the blood vessel lumen and is largely

composed of proteoglycans and GAGs. Within the glycocalyx, ECM components close to the endothelial surface (within 50 nm) facilitate interactions with endothelial adhesion molecules on circulating leukocytes. This structure of the glycocalyx raises a key challenge to our current understanding of leukocyte migration. Specifically, how can leukocytes interact with endothelial adhesion molecules when they are covered by a glycocalyx "blanket" that is too thick to be simply bridged by endothelial and leukocyte adhesion molecules (Fig. 2) (*15*, *17*, *18*)? Enzymatic digestion of glycocalyx components has been shown to reveal endothelial adhesion molecules and facilitate increased leukocyte–endothelial interactions (*15*, *16*, *19*). Degradation of the glycocalyx is a critical feature of sepsis, where TNF is a key inducer of endothelial heparanase, leading to exposure of adhesion molecules and facilitating neutrophil trafficking (*20*, *21*). However, the role of the endothelial glycocalyx in trafficking of leukocytes other than neutrophils is poorly understood. Identification of glycocalyx fragments in the serum of patients with sepsis, cardiovascular disease, and more recently COVID-19 (*19*, *22–24*) suggests that breakdown of the glycocalyx likely contributes to disease. However, a caveat is that these fragments may be released from sources other than the endothelium because the specific glycocalyx content and structure across different vessel types and tissues is not well characterized. A deeper analysis of glycocalyx structures throughout the body will enable us to better understand the consequences of its degradation during inflammation.

The matrix as a guide to immune cell movement and position

Multiple physical and chemical properties of the ECM guide cell migration (*25*). In numerous settings, leukocytes have been shown to navigate via the path of least resistance often through "holes" in the matrix that have been described extensively (*26*, *27*). For example, T cells will

preferentially move through tissue sites that are characterized by thin fibers of collagen and fibronectin, deliberately avoiding denser matrices that are made more substantial by lysyl oxidases (LOX) that cross-link collagen proteins (*28*). The inhibition of LOX, which weakens the fibrillar collagen network, improves T cell access to tumors (*29*) and increases the speed and travel distance of innate lymphoid cells (ILCs) during lung inflammation (*30*). Moreover, collagen fibers can directly influence cell motility as shown by the ability of type 1 collagen to alter the cytoskeletal organization and shape of ILCs toward a promigratory exploratory phenotype (*30*). Equally in inflamed skin, confirmational changes to fibronectin fibers result in enhanced integrin binding. These altered ECM fibers can arrest interstitial migration of T cells, causing cellular accumulation at perivascular sites (*31*). The mechanical stretching properties of native ECM fibers can further regulate integrin-mediated cell binding and biochemical signaling (*32*). Once positioned within a tissue environment, the necessity for communication with the ECM has been highlighted by the identification of key receptors for ECM on a variety of myeloid cell subpopulations. LAIR1, a receptor that binds glycine-proline-hydroxyproline repeats in collagen, is needed for the survival, proliferation and differentiation of monocytes and interstitial macrophages in the lung (*33*).

Hyaluronan (HA), a major structural component of the ECM, is an enormous hydrophilic GAG (greater than $10^6$ Daltons) made up of unbranched repeating units of D-N-acetyl glucosamine and D-glucuronic acid. Through interactions with its receptors, HA facilitates immune cell function in part by directing localization. HA is found in the glycocalyx of many cells. In some cases, such as the alveolar macrophage, it is constitutively bound to the cell surface through CD44, an HA receptor found on most leukocytes (*34*). With a rapid turnover compared to other ECM components (*35*, *36*), HA production is tightly balanced and controlled locally through CD44-mediated cellular uptake and degradation by hyaluronidases (reviewed in (*37*)). HA–CD44

112 interactions can be essential for leukocyte migration and location, as shown in the HA-rich liver sinusoids where CD44 binding to HA allows neutrophil adhesion in the absence of other adhesion molecules (*38*). Following antigen-induced activation T cells will bind HA through enhanced expression of CD44 (*39*), possibly honing their recruitment to specific inflamed tissue sites (*40*).
116 Lyve-1, another receptor for HA, is found on macrophages that have specific functions in maintenance of the vasculature. Here, HA dictates Lyve-1$^+$ macrophage location along the blood vessel wall (*41*). Additionally, Lyve-1 expression on the lymphatic endothelium acts as a dock allowing dendritic cells (DCs)—and potentially other immune cells that are endogenously coated
120 in HA—to adhere and traffic through the lymphatics (*42*). It is not simply the presence of HA and its receptors that determine cellular location but the specific composition of the HA matrix. For example, Lyve-1 only binds HA that is correctly organized and crosslinked by molecules such as TSG-6, which is upregulated during inflammation (*43*). Additionally, versican, a large chondroitin
124 sulfate containing proteoglycan, can bind to HA with high affinity forming distinct HA matrices that often accumulate during inflammation (*44*). TSG-6 cross-linking of HA (Fig. 3) and versican–HA aggregates can also enhance cellular interactions with CD44 (*45*, *46*) adding another level of ECM control to leukocyte adhesion and/or migration. Moreover, HA receptors can act in tandem.
128 CD44 alters the spatial organization of the HA glycocalyx on DCs to allow binding to Lyve-1 on the lymphatic endothelium regulating the efficiency of DC entry to the lymphatics (*47*).

Similarly, heparan sulfate (HS) proteoglycans help direct recruitment and positioning of neutrophils and monocytes via direct interactions between their GAG side chains and P and L
132 selectins on immune cells (*48*, *49*). The ECM can also act indirectly as a cell guide by "presenting" interacting proteins. For example, HS proteoglycans bind and present chemokines within the vasculature as well as the tissue parenchyma to form gradients that direct cell movement (*50*, *51*).

This interaction is also important in decoding complex chemokine signals. For example, the CXCR3 ligands CXCL9, CXCL10, and CXCL11 are often expressed together but have very different affinities for HS, which allows discrete localization (*52*). HS proteoglycans also bind cytokines but the consequences for immune cell location and function of these interactions remain relatively unexplored.

The inflammation-altered matrix

A fundamental feature of the ECM is that it is altered following inflammation, injury, or infection and with age (*53–55*). For example, HA increases in the lung following flu infection (*55*) and during aging (*54*). Many different molecules bind to HA chains forming large, hydrated matrix scaffolds (Fig. 1C and Fig. 3). During injury and inflammation, HA can become covalently modified with heavy chains (HCs) from the inter-alpha-inhibitor (IaI) family of proteoglycans (*56*) forming crosslinked HA matrices mediated by HC–HC and pentraxin–HA interactions (*57*, *58*) (Fig. 3). Although such HC•HA matrices are critical for some normal biological processes, they are generally only made in tissues during inflammatory conditions. Crosslinked HC•HA can have increased affinity for CD44 and binding to leukocytes (*59*, *60*), which can lead to retention of immune cells within the pathogenic tissue ECM prolonging inflammation (*61*). However, the contribution of a HA matrix to any immune process will depend on its composition and the specific tissue or disease context (*56*). These large hydrated HA structures, including HA bound to versican, can also directly restrict cell movement. Two matrix proteases, ADAMTS4 and ADAMTS5, are both needed during flu infection to breakdown versican, remodeling the ECM such that a conduit is created through which $CD8^+$ T cells can travel (*62*, *63*). The failure to provide

passage for $CD8^+$ T cells in *Adamts4*-deficient mice (*62*) prevents immune pathology but compromises host protection in *Adamts5*-deficient mice (*63*).

Breakdown of the ECM by proteases such as the ADAMTS family members generates fragments that are themselves immunoregulatory (reviewed in (*44*)). For example, HA becomes fragmented at sites of inflammation and injury leading to the buildup of low-molecular-weight HA (LMW-HA), which may antagonize normal HA functions through competition for receptor binding (*64*). Although the specific immunoregulatory roles of LMW-HA remain to be elucidated, *Cd44*-deficient mice, which fail to clear LMW-HA, die from lung failure following bleomycin induced fibrosis (*65*). Consistent with the role of HA in regulating immune cell function, HA binding via the CD44 receptor on activated regulatory T cells (Tregs) promotes IL-2 production, persistence of FoxP3 expression, and enhanced IL-10 production (*66*, *67*). Other ECM components released by tissue damage have well-established roles as "danger signals" (*68*–*70*). Toll-like receptors (TLRs) 2 and 4, in particular, recognize proteoglycans such as biglycan, aggrecan and versican as well as various GAGs. Macrophages can be activated via TLR2 and TLR4 by heparanase-mediated GAG cleavage and this is associated with atherosclerotic plaque progression (*71*). Given that ECM will be one of the first tissue components damaged by infection, it makes sense that its degradation acts as a danger signal to the immune system.

The ECM is a rich source of chemokines, cytokines, and growth factors, whose availability is controlled by the ability of immune cells to access these factors. Indeed, many immune cells including T cells express heparanase, which digests HS to release bound cytokine (*72*). However, damage or remodeling of the ECM caused by tissue injury or matrix-degrading enzymes can also

cause the release or activation of cytokines normally bound to GAGs (*73*). The bioavailability of TGF-β, one of the most important cytokines in the regulation of the ECM, is itself tightly controlled by its regulated release from the ECM (*73*). The mechanical state of ECM fibers such as their capacity to stretch or unfold can also dictate cytokine availability or activity (*32*). For example, the large latent TGF-β complex stored in the ECM can be activated by mechanical forces, and stiffer matrix can reduce the threshold for TGF-β1 activation (*74*). Thus, the capacity of inflammation to alter the chemical composition and mechanical properties of the ECM will modify an enormous range of immune processes and needs to be considered when investigating the initiation or resolution of an inflammatory response.

Mechanical changes with age and disease

Many tissues lose mechanical compliance with age and during the course of fibrotic disease. Although alterations to the fibrillar collagen network are a major determinant of pathological tissue stiffness, other ECM changes alter the mechanical properties of the ECM with age and disease (*75*) including increased HA (*54*, *55*, *61*). Notably, the transcriptomes of tissue resident alveolar macrophages change with age not because of the age of the cell but due to signals from the aging lung ECM (*54*). The ability of immune cells to respond to mechanical cues is now well established (*76*) and many (if not most) fundamental immune processes will likely be influenced by the biophysical properties of the matrix environment. For example, an emerging paradigm suggests that stiffer, less elastic tissues promote an inflammatory response in macrophages (*77*). However, numerous signals integrate to determine macrophage activation state and more compliant substrates can increase sensitivity to pro-inflammatory stimuli (*78*). Because the majority of work on this topic has involved either in vitro analysis or the use of biomaterials in vivo, we still lack an understanding of the contribution of natural tissue-specific variation in ECM elastic and tensile

properties (e.g, soft brain and hard bone). The remodeling of the ECM following injury, such as the deposition of thicker and denser collagen fibrils in scar tissue, is strongly implicated as a driver of further disease progression (*2*, *79*). However, the unique biophysical properties of native ECM fibers are very difficult to replicate synthetically (*32*) and thus the specific functional consequences for local immune cells remain to be discovered.

Notably, an intimate link between mechanosensing of the ECM and regulation of inflammatory signals can drive ageing (*80*). The transcriptional activators YAP and TAZ transduce mechanical signals and their activity declines with physiological ageing in stromal and contractile cells. YAP–TAZ signaling inhibits the proinflammatory immune cGAS–STING pathway and when this restraint is lifted, cell senescence proceeds with the emergence of ageing-related tissue degeneration (*80*). The identification of specific receptors involved in mechanotransduction is also providing new understanding of the impact of tissue biophysical properties on immune cell function. For example, Piezo1, a mechanosensitive ion channel protein, has been demonstrated in vivo to regulate macrophage responses to physical forces (*81*). More broadly there is good evidence that Piezo1 regulates immune cell function (*82*, *83*). Critically, this is a two-way communication: while tissue stiffness regulates immune cell function, immune signals can alter tissue stiffness in a wide variety of mechanisms from regulating collagen production and cross-linking (see below) to inflammation induced HA matrices (*56*).

## How do immune cells regulate ECM composition?

ECM regulation by chemokines and cytokines

The cytokine with the most evident influence on shaping the ECM is TGF-β, with its ability to promote myofibroblast differentiation, induce collagen production and inhibit the matrix metalloproteinases that degrade matrix (*75*). However, type 2 cytokines and in particular IL-13 have emerged as another layer of cytokine control over matrix quantity and quality of the ECM. Consistent with type 2 cytokines as mediators of tissue repair and remodeling (*84*), type 2 cytokines regulate the production of collagen degrading enzymes (*85*), the production of collagen by macrophages (*86*), and collagen uptake and degradation (*87*). IL-4 and IL-13, which both signal through the IL-4 receptor alpha (IL4Rα), appear to be central players in the maintenance of the ECM, but also in ECM dysregulation. IL4Rα signaling induces dermal, synovial and lung fibroblasts to produce more collagen, as well as hepatic stellate cells, which are the primary source of aberrant ECM during liver fibrosis (*88–93*). Indeed, IL-13 signaling to fibroblasts is sufficient to drive liver fibrosis (*94*). IL4Rα signaling can also regulate the amount of collagen-cross linking through signaling to macrophages. IL4Rα-dependent RELMα production by macrophages is critical for vascular integrity and repair of the skin through regulation of collagen cross-linking enzyme lysyl hydroxylase in fibroblasts (*95*). In addition to effects on collagen during ECM remodeling, IL-13 signaling can directly induce hyaluronan accumulation in the lungs, a key mechanism recently identified as a driver of severe COVID-19 pathology (*96*).

While the finding that IL-13 regulates hyaluronan is new (*96*), it is well documented that IL-13 regulates mucus, another sugar-rich “gel-like” secreted ECM structure. IL-13-driven goblet cell

hyperplasia and enhanced mucus production are essential steps in protection against gastrointestinal nematodes (*97*). However, less well appreciated is that IL-13 alters not only the quantity but the composition of the mucus with consequences for disease outcome. For example, IL-13 specifically induces the mucin Muc5AC, whose overproduction is an essential feature of allergic airway inflammation (*98*) as well being required for nematode expulsion (*99*). IL-13 also increases mucin sulfation, which makes it more difficult for the helminth *Trichuris muris*, to establish a successful infection (*100*). Perhaps the most profound impact of immune alterations to the mucus barrier is the key role sugars play in regulating the microbiome. Host GAGs and mucins are food sources for commensal bacteria and the ability of bacterial species to utilize these sugars will regulate the gut microbial composition (*101*). For example, *Bacteroides theatiotaomicron* produces sulfatases that enable it to utilize specific mucins (*102*) and metabolise GAGs (*103*). In an elegant example of this interaction, the opportunistic bacteria *Pseudomonas aeruginosa* induces a type 2 immune response in the lung stimulating Muc5AC production, which the bacteria then uses as an energy source to enhance colonization. This type 2 enhancement loop additionally results in allergic sensitization (*104*).

Cytokines such as TGF-β and IL-13 regulate the ECM by driving ECM production or enzymatically regulating its composition, whereas direct biophysical interaction with chemokines or cytokines can alter ECM structure and/or function. Some proteins are thought to bind to GAGs via less-specific electrostatic driven interactions, whereas others bind more specifically based on shape complementarity (*5*). The nature of these protein–GAG interaction may dictate functional biological outcomes. For example, chemokines display highly variable affinities for GAGs (*105*, *106*), suggesting distinct biological functions of these interactions that go beyond using GAGs as

a platform for a chemotactic gradient (*48*). Although it makes sense that weaker (less-specific) interactions will facilitate localization, particularly in the context of the bloodstream, certain chemokines (e.g., CXCL4 (PF4)) bind with incredibly high affinity. Recent work has suggested that some chemokines like CXCL4 primarily function by binding to GAGs, rather than directly to chemokine receptors (*107*). This can be mediated by signaling through cell surface proteoglycans and/or remodeling the ECM glycocalyx to facilitate leukocyte–endothelial interactions (*108*). For instance, high-affinity binding by CXCL4 leads to the cross-linking of HS chains resulting in shrinking of the thickness of the glycocalyx (Fig. 2). This reduced depth may allow better cellular access to the endothelial surface. In addition to chemokines, a number of cytokines and growth factors have been shown to bind to and remodel GAGs, such as IFN-γ whose function is regulated by this interaction (*109*). We still have much to learn about the mechanistic role of cytokine–GAG interactions particularly to better understand how GAGs control cytokine availability and/or present cytokines to their receptors.

ECM remodeling by immune cells

Immune cells typically migrate using non-proteolytic mechanisms which ensure that matrix barriers are not catastrophically compromised. However, to transverse the rigid basement membranes, activated macrophages utilize membrane-type I matrix metalloproteinase (MT1-MMP) as well as cytoskeletally generated physical forces (*110*). Notably, neutrophils can be found coated in laminin after passing through holes in the basement membrane suggesting a level of localized remodeling (*111*). Additionally, without expression of elastase, neutrophils cannot breach the vascular basement membrane and migrate into the interstitial matrix (*112*), which may also impact on subsequent migration of other cells like eosinophils (*113*). Indeed, although matrix-

degrading enzymes involved in cell trafficking can be produced by resident stromal fibroblasts (*62*), they are often made by the immune cells themselves. During flu infection, neutrophil-derived MMP9 is required for neutrophils to access the infection site and to control viral replication (*114*), whereas ECM degradation by MT1-MMP, highly expressed on infiltrating myeloid cells, compromises tissue integrity and promotes secondary bacterial infection (*115*). These studies highlight the possibility that "pioneer" cells recruited early in recruitment can modify the ECM to facilitate and control subsequent waves of immune cells, consistent with the known role of neutrophils in directing T cell migration (*116*). However, myeloid cells are not alone in their capacity to remodel the ECM as T cells with intrinsic loss of granzyme B (which degrades several ECM components) have reduced extravasation in vivo (*117*). These studies highlight inflammatory events that involve local remodeling of the basement membrane allowing other cells to gain access into the tissue. However, the capacity of neutrophils to remodel the ECM goes beyond enzyme release. Remarkably, in the early stages of tissue repair, neutrophils use cell surface integrins to pull and carry pre-existing matrix from nearby tissue where it is incorporated into the wound-bed matrix to re-establish a new ECM scaffold (*118*).

Macrophage remodeling of the ECM

Beyond the secretion of collagen-degrading enzymes, macrophages play a pivotal role in mature ECM turnover via receptor mediated uptake of collagen and routing to lysosomes for degradation (Fig. 4). Enhanced collagen turnover occurs via the upregulation of members of the mannose receptor family, which target specific collagen types for degradation, in a process enhanced by type 2 cytokines (*87*). However, in some contexts, collagen scavenging by macrophages can enhance rather than limit collagen deposition. The superabundance of intracellular arginine

generated by collagen breakdown promotes a metabolic switch toward reactive nitrogen species, which triggers further collagen deposition (Fig. 4) (*119*).

Critically, macrophages are not just mediators of matrix removal, but directly produce collagen themselves. Myofibroblasts are typically the major producers of fibrillar collagen during scar formation and ECM remodeling and the functional purpose of macrophage collagen production remains unclear. In models of heart injury, macrophages produce collagen that contributes to scar formation (*120*). This was observed in both mice and zebrafish suggesting that collagen deposition is an evolutionarily conserved property of macrophages, further supported by evidence of collagen production by macrophages in *Drosophila* (*121*). Macrophages activated by numerous signals including IL-10, IL-13, and/or TGF-β secrete collagen VI (*86*), a molecule that binds different ECM components and plays a role in organizing 3D tissue architecture (*122*). Although the specific role for macrophages versus fibroblasts is not yet apparent, macrophages produce several ECM components (e.g., fibronectin, laminin, osteopontin, and versican) and may provide an initial scaffold following injury before the fibroblasts take over. In a pancreatic cancer model, macrophages produce a subset of collagen isoforms distinct from fibroblasts providing evidence that macrophages fine-tune the fibrotic response (*123*). However, in a colorectal cancer model, collagen producing macrophages actually outnumbered the fibroblasts and were directly responsible for the remodeling of the tumor microenvironment (*124*).

The past decade has seen a deepening expanse of knowledge demonstrating critical tissue-specific functions of resident macrophages that act to maintain tissue integrity and identity (*125*). It seems very likely this will include the ability to define the unique composition of the different ECM

niches. Indeed, the intimate mechanistic relationship between particular macrophage subsets and fine tuning of collagen matrix is now being revealed. For example, Lyve-1$^+$ macrophages, which localize to the collagen-rich adventitial layer of large blood vessels, are able to degrade collagen 1 produced by vascular smooth muscle cells (SMC), primarily via MMP-9 release (*41*) (Fig. 4a). This control of collagen production requires Lyve-1 binding to HA on the vessel wall and is needed to prevent the ECM remodeling and arterial stiffness associated with cardiovascular disease. The regulation of ECM turnover by Lyve-1$^+$ macrophages is likely to apply more broadly as suggested by the ability of Lyve-1$^+$ macrophages to regulate both collagen and HA levels in the mammary gland (*126*).

**Summary**

Although this review is divided into matrix impact on immune function and vice versa, the reality is naturally more complex and many of the examples we describe reflect an active cross-talk between matrix components and the immune system. For example, implanted ECM scaffolds made from decellurized porcine tissue induce a type 2 immune response (*127*, *128*), illustrating the capacity of ECM alone to regulate an immune response. In turn, the type 2 response it elicits, promotes an eosinophil and macrophage program that helps drive ECM deposition (*84*). Development of targeted therapies for any immune-mediated condition must therefore consider both the immune cell and the physical environment that will surround it. Advances in techniques to spatially map where immune cells preferentially reside within the tissue are transforming our understanding of cell–cell interactions (*129*). However, to truly understand how immune cells behave and function in a healthy versus diseased or aging tissue, these methodologies need to incorporate the complex and changing ECM structures that define the tissue niche, which will

include tackling the glycome (*130*). Ultimately, to answer the most pressing questions in tissue health, it will be critical that immunologists and matrix biologist work together.

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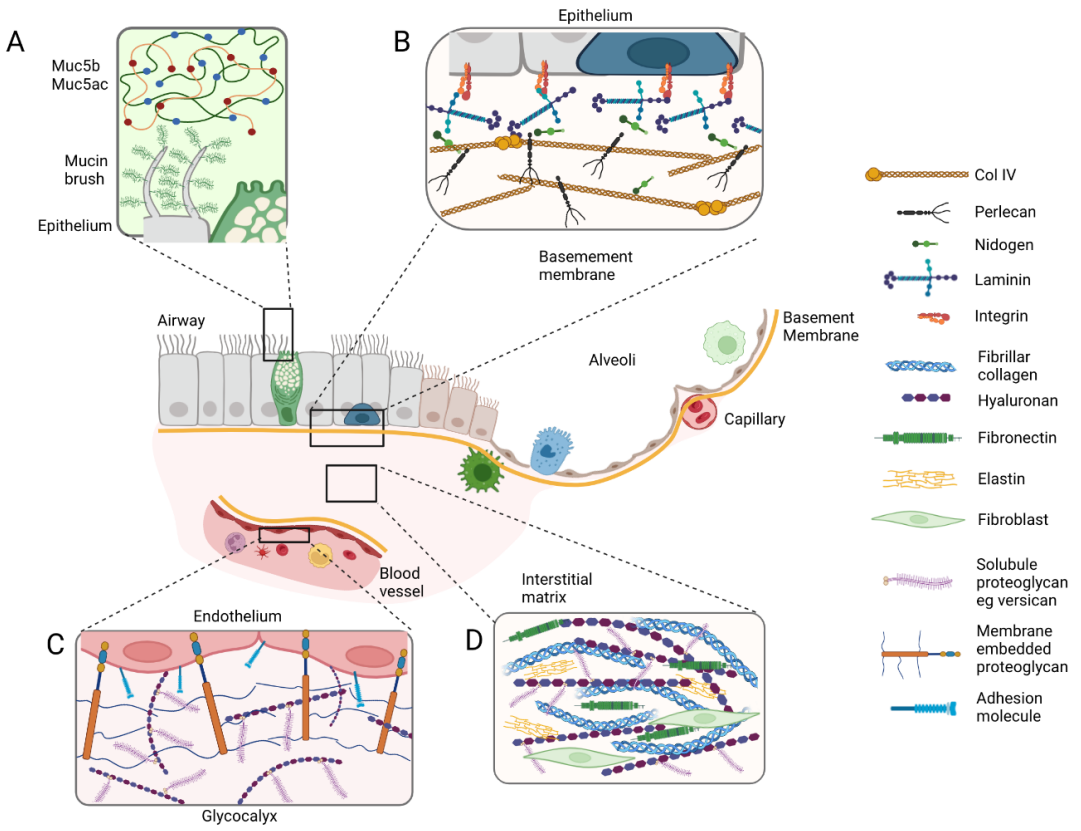


**Fig. 1. Extracellular matrices in the healthy lung.** Overview of a healthy lung airway and alveolar structure, including capillaries, the blood vessels and the various extracellular matrices that can be found within the tissue architecture. (**A**) Airway epithelial cells are protected by a periciliary layer containing mucins that are tethered to the cilia of epithelial cells and a gel-like mucus mesh of secreted mucins Muc5ac and Muc5b. (**B**) Basement membrane (BM) ECM forms a sheet-like layer that coats the basal surface of epithelial and endothelial cells. Collagen IV fibers are important for maintaining normal architecture of the tissue. Laminin chains form into a cruciform structure linking cells through integrin binding to the BM and connect to collagen fibres directly or through interactions with heparan sulphate proteoglycans (e.g., perlecan) and proteins (e.g., nidogen), overall contributing to an ECM network. (**C**) The glycocalyx is a brush-like layer that lines the luminal surface of endothelial cells and is rich in membrane-bound proteoglycans such as syndecan, glycosaminoglycans such as hyaluronan (HA), and soluble chondroitin sulphate proteoglycans like versican. (**D**) The interstitial lung matrix is primarily maintained by fibroblasts and made up of core proteins type I and III

fibrillar collagens, and elastin arranged in a 3D network providing structural support and flexibility of the lung during homeostasis. Fibronectin helps orientate new collagen fibers and can aid cell anchoring to the ECM through integrin binding. HA—the largest and most abundant non-sulphated GAG—helps assemble, hydrate, and stabilize connective-tissue ECM and can be decorated with proteoglycans such as versican. Molecules are not shown to scale.

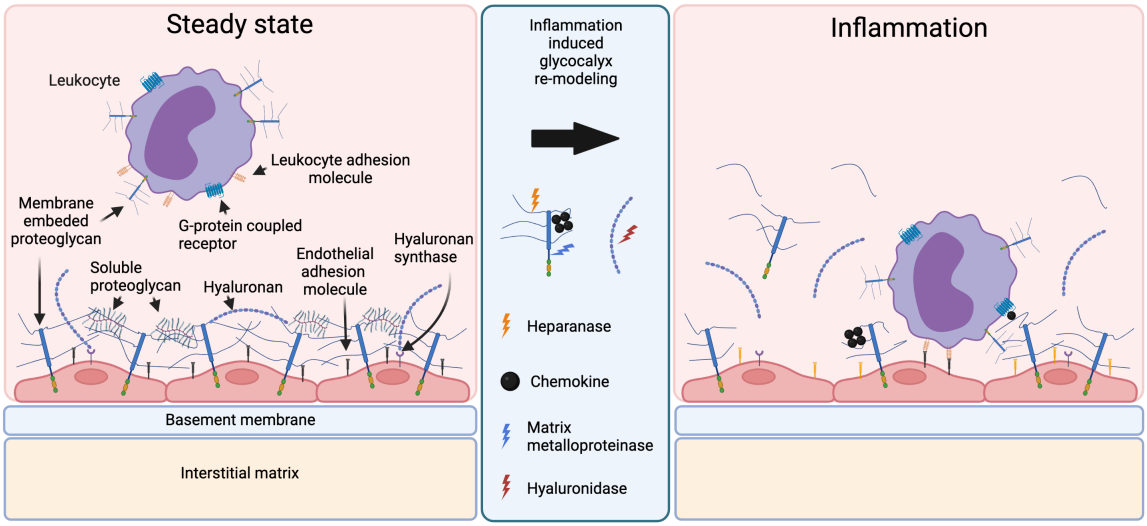


**Fig. 2. The endothelial glycocalyx shield is remodeled during inflammation to facilitate leukocyte rolling on the endothelium.** The endothelial glycocalyx is a thick (200-2000 nm) barrier on the blood exposed endothelial surface largely composed of extracellular matrix proteoglycans. This barrier forms a "blanket" over the endothelial adhesion molecules (e.g., P-selectin) blocking interaction with adhesion molecules on circulating leukocytes (e.g., PSGL1) and preventing aberrant leukocyte recruitment. Following an inflammatory stimulus (e.g., TNF), endothelial cells and leukocytes themselves, can produce factors that remodel the glycocalyx barrier to facilitate endothelial–leukocyte interactions. Specifically, heparanse cleaves the GAG sugar side chains of proteoglycans, matrix metalloproteinases cleave their protein cores and hyaluronidase cleaves hyaluronan, increasing their levels in the circulation. Additionally, chemokines may cross-link GAG chains so that they occupy less space within the glycocalyx. Together these mechanisms enable leukocytes to access the endothelial surface, initiate rolling, and subsequently enter underlying tissues.

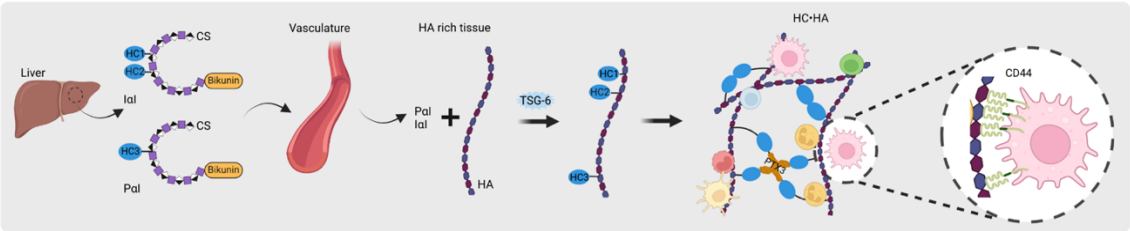


**Fig. 3. Heavy chain crosslinked hyaluronan matrix.** Inter-alpha-inhibitor (IαI) and pre-IαI (composed of bikunin and heavy chain (HCs) proteins attached to a chondroitin sulfate backbone) are assembled in the liver and secreted into the circulation with transfer of HCs during inflammation catalysed by tumour necrosis factor-stimulated gene 6 (TSG-6). Formation of HC•HA leads to cross-linking of HA chains via interactions with HCs and via HCs with accessory proteins such as pentaxin 3 (PTX3). Cross-linked HC•HA can stabilize a pathological HA matrix that has enhanced affinity for HA receptor CD44 and hence increased adhesion for leukocytes.

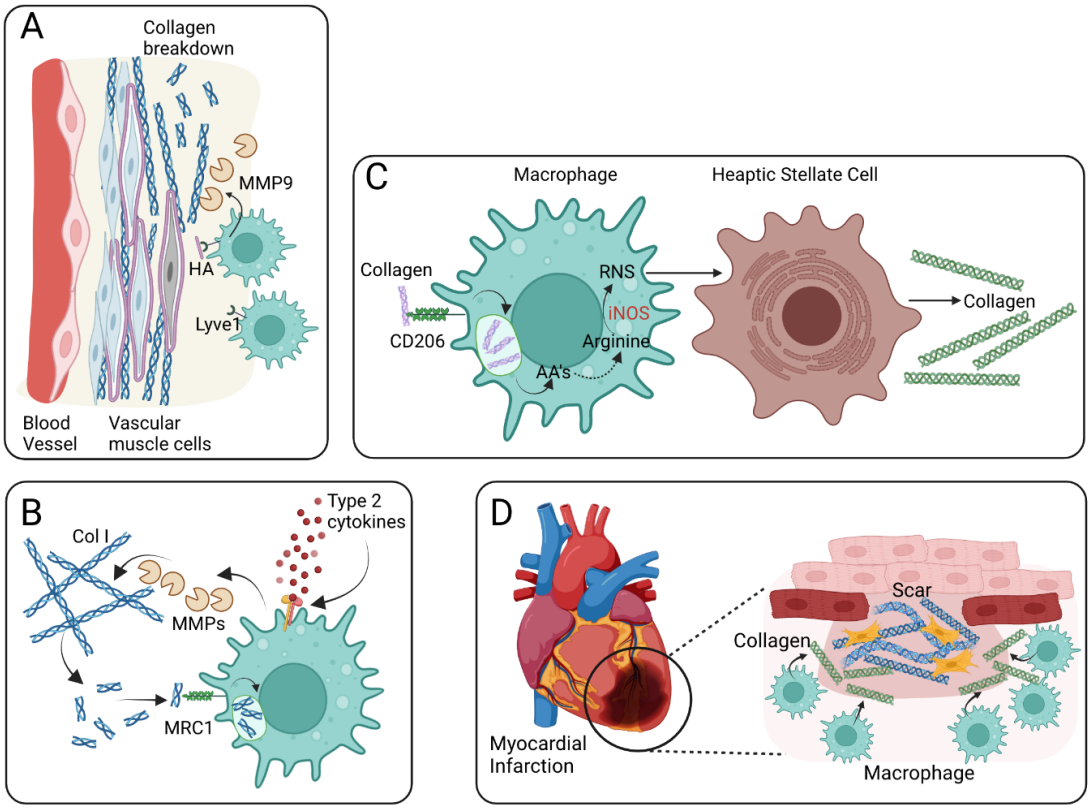


**Fig. 4. Macrophages regulate collagen production and breakdown.** (**A**) Macrophages located around the outer region of the blood vessel wall express Lyve1, a receptor which recognizes and binds hyaluronan, a component of the extracellular matrix that can be found on the pericellular coat of cells such as vascular smooth muscle cells. This Lyve1–HA interaction initiates MMP9 mediated proteolysis of collagen fibers and thereby regulates the composition of the arterial ECM and hence maintains tone and diameter of the artery. (**B**) Collagen degradation by MMP9 also occurs following macrophage activation by type 2 cytokines. Fragments of collagen bind through mannose receptor (MRC1) and are taken up intracellularly into lysosomes within the macrophage, where it is further processed for degradation (*87*). (**C**) Collagen degradation mediated through MRC1 signaling can lead to an increase in free amino acids (AAs) intracellularly, which drives arginine biosynthesis and subsequent generation of reactive nitrogen species (RNS) through iNOS activity. Release of RNS has been shown to activate profibrotic pathways in pancreatic stellate cells that results in collagen synthesis (*119*). (**D**) Macrophages themselves have also been shown to directly produce different collagen

molecules. During myocardial infarction, macrophages at the site of injury contribute to deposition of collagen specifically at the peripheral of the scar (*120*).