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Towards integrating extracellular matrix and immunological pathways

David F. Boyd, Paul G. Thomas *

Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN 38105, United States

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ABSTRACT

The extracellular matrix (ECM) is a complex and dynamic structure made up of an estimated 300 different proteins. The ECM is also a rich source of cytokines and growth factors in addition to numerous bioactive ECM degradation products that influence cell migration, proliferation, and differentiation. The ECM is constantly being remodeled during homeostasis and in a wide range of pathological contexts. Changes in the ECM modulate immune responses, which in turn regulate repair and regeneration of tissues. Here, we review the many components of the ECM, enzymes involved in ECM remodeling, and the signals that feed into immunological pathways in the context of a dynamic ECM. We highlight studies that have taken an integrative approach to studying immune responses in the context of the ECM and studies that use novel proteomic strategies. Finally, we discuss research challenges relevant to the integration of immune and ECM networks and propose experimental and translational approaches to resolve these issues.

1. Introduction

Immune responses to infection and injury are often tissue-specific. Migration, proliferation, and differentiation of immune cells depend on cytokines and growth factors that accumulate in the tissue microenvironment. The extracellular matrix (ECM) is a major component of any tissue and helps define its structure and function. Disruptions and alterations in the ECM feed into immunological pathways, which in turn regulate repair and regeneration of the ECM. The ultimate outcome of these regulatory circuits determines whether the tissue regains adequate function in a manner supportive of host recovery. Here we review the evidence that the ECM plays a critical role in modulating tissue-specific immune responses to infection and injury. We will primarily drawn on examples from the lung, an organ with an extensive extracellular matrix that is constantly remodeled in response to infection and other insults. We will describe the major components that make up the ECM structure, enzymes that are involved in remodeling the ECM, and cytokines and growth factors associated with the ECM that modulate host immune responses. We propose a unified theory of immunology and ECM biology in which host immune responses to infection and injury are carried out in the context of the ECM. For many diseases, persistent inflammation is associated with poor outcome. Given the important role that the ECM plays in modulating inflammation mediated by the immune system, improved understanding of the basic mechanisms underlying these interactions will inform the development of therapeutics that seek to limit immunopathology and promote restoration of tissue function.

2. ECM proteins

The extracellular matrix is a complex and dynamic structure made up of an estimated 300 different proteins in mammals [1]. The ECM can be generally divided into two main components: the interstitial matrix and the basement membrane. Collectively, these ECM proteins are often referred as the matrisome. In addition to providing structural support to all tissues, the ECM plays a critical role in most basic cellular functions, including differentiation, migration, proliferation, and turnover. Generally, these ECM proteins can be segregated into broad, diverse groups of collagens, proteoglycans, and other complex ECM glycoproteins. The majority of proteins that have been identified in the ECM belong to the 'other' category, highlighting the need for more research to better define their functional roles.

ECM genes are evolutionarily ancient, and a core set of genes that encode proteins in basement membranes likely existed in basal metazoans [1]. Comparative genetic analysis of vertebrate genomes with those of closely related invertebrates, suggest that when the vertebrate lineage diverged there was a dramatic expansion in the number of ECM genes and corresponding remodeling enzymes [2,3]. This expansion appears to mostly be the result of gene duplication and subsequent diversification [1,2]. The evolution of the complex mammalian immune system, including both innate and adaptive branches, occurred after this expansion of

* Corresponding author.
E-mail address: Paul.Thomas@stjude.org (P.G. Thomas).

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ECM-related genes [4]. Thus, the mammalian immune system and all of its constituents, including many innate and adaptive immune cell types, soluble mediators, and molecular effectors, has developed in the context of this dynamic and diverse extracellular matrix structure. In addition to using signals from the ECM to coordinate host responses to infection and injury, immune cells play an active role in remodeling the ECM and promoting tissue repair.

In the following section, we outline the major groups of ECM proteins and highlight important functions of specific proteins within each. For in-depth analysis of these groups of ECM proteins, we refer readers to an excellent review of the matrisome by Hynes and Naba [1].

2.1. Collagens

Collagens confer tensile strength to the ECM of tissues and are characterized by the presence of Gly-X-Y repeats, where X and Y can be any amino acid, but are frequently proline and hydroxyproline [5]. Through homotypic interactions between these repeats, collagens form stable, trimeric structures. These trimeric structures also form higher order oligomers that contribute to the strength of the ECM. Impressively, 28 different types of collagen have been identified in vertebrates [6]. Fibrillar collagens, including types I and III, are predominantly found in the interstitial matrix. In contrast, network forming collagens, including type IV, are found in the ECM basement membrane and provide a rigid surface for epithelial and endothelial cells [5,6].

2.2. Proteoglycans

Proteoglycans are glycoproteins that contain repeating glycosaminoglycans (GAGs). These disaccharides have attached carboxyl and sulfate groups that confer a strong negative charge to the molecule. Due to these GAGs, proteoglycans are able to bind numerous cytokines and growth factors and retain them in the ECM [7]. Approximately 36 ECM proteoglycans have been identified in mammals, and these proteins have diverse functions in multiple different tissues [1,8]. For example, the proteoglycan hyaluronan is abundant in the lung and plays a major role in maintaining tissue homeostasis and in responding to lung injury [9]. Another proteoglycan, versican, associates with hyaluronan to form long filaments in the ECM [10]. These filaments have been demonstrated to play an important role in modulating inflammatory responses to infection and tissue injury and in immune cell adhesion and migration [11]. Proteoglycans are found in both interstitial and basement membrane matrices. While hyaluronan and versican are localized in the interstitial ECM, the heparin sulfate proteoglycan perlecan is found in the basement membrane and is critical for its formation across many species [12]. Interestingly, hyaluronan has been demonstrated to engage innate immune sensors present on epithelial cells in the lung [13–15]. A recent study showed that hyaluronan engagement of toll-like receptor-4 (TLR4) promoted renewal of alveolar progenitor cells and tissue repair, preventing lung fibrosis. In TLR-4-deficient mice, bleomycin-induced injury was exacerbated and tissue repair was compromised due to impaired renewal capacity of type 2 alveolar epithelial cells. Taken together, these studies indicate that proteoglycans in the lung ECM interact with innate immune sensors to regulate tissue-repair mechanisms highlighting the important interplay between immunity and the ECM.

2.3. Other ECM glycoproteins

In addition to the collagens and GAG containing proteoglycans, there are numerous other complex proteins that have been identified in the ECM. Unbaised approaches to defining the matrisome have identified approximately 200 of these proteins that comprise a diverse set of molecules that mediate ECM-cell interactions, cell signaling, and binding to growth factors, among other functions. Another main function of these proteins is to serve as linkers in the ECM connecting other ECM proteins and helping to define the structure of a tissue. Laminins, present in basement membranes, and fibronectin, found in the interstitial matrix, are among the most abundant and well studied glycoproteins in the lung ECM [16]. Although hundreds of ECM glycoproteins have been identified, few of them have been extensively studied, and their roles in homeostasis and in disease are largely unknown. As we gain better understanding of the functional roles of these additional ECM proteins, a more narrow and informative categorization of this diverse group of molecules will be possible.

As previously mentioned, the ECM can generally be divided into the interstitial matrix and the basement membranes. The interstitial matrix is made up primarily of fibrillar collagens and fibronectin and serves as a scaffold to the tissue. In the lung, elastin, an ECM glycoprotein, is a major component of the interstitial matrix. The basement membrane, composed primarily of type IV collagen and laminins, is a more rigid and compact ECM structure that interacts directly with epithelial and endothelial cells [17]. The compact network of the basement membrane poses a potential barrier to the infiltration of immune cells surveying the tissue or responding to infection or injury. Although protease-mediated degradation of the basement membrane has been proposed as an important factor in promoting extravasation of immune cells into the interstitium, this process would involve the disruption of an extensive ECM network [18]. An alternative hypothesis has been proposed in which variability in the composition of the basement membrane determines extravasation of immune cells at sites with a lower density of ECM proteins. In support of this idea, neutrophils and monocytes have been demonstrated to preferentially migrate through areas of low collagen IV expression in an *in vivo* mouse model [19,20].

Recently, there have been efforts to characterize the matrisome in an unbiased and comprehensive manner across different tissues using proteomic approaches. These studies have attempted to quantify the ECM in a variety of tissues ranging from the lung and heart to bone and cartilage and across different species, including humans, mice, and pigs [21]. Interestingly, a study by Naba et al. comparing the ECM composition of lung and colon tissue in mice found that approximately 10–30% of the ECM proteins are tissue-specific [16]. As previously mentioned, the ECM of a given tissue is highly dynamic both during homeostasis and in pathologic conditions. Several groups have begun to assess ECM composition temporally over the course of certain disease states, particularly in the lung. For example, Decaris et al. characterized the alterations in ECM turnover following bleomycin-induced lung fibrosis, and Talmi-Frank et al. measured changes in lung ECM protein abundance in the context of influenza infection [22,23]. Proteomic analysis of samples after differential extraction and from different soluble fractions has the potential to provide improved resolution of the matrisome based on lung compartment [24].

Another disease state in which there are dramatic changes in ECM composition is cancer. Changes in ECM composition can help generate microenvironments conducive to tumor cell growth [25]. For example, in a murine model of lung cancer cell metastasis, fibronectin is upregulated in future metastatic niches [26]. Just as the ECM plays an important role in normal cell migration, it also influences cancer cell motility. Enzymes that remodel ECM proteins, which will be discussed below in more detail, facilitate cancer metastasis by permitting migration of these cells across extracellular matrices and into distant tissues [17]. Recently, proteomics approaches have also been used to analyze the ECM in tumor microenvironments, in particular for colorectal cancer.

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