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Linking Immune Responses with Fibrosis in Allergic Eye Disease

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Abstract

Purpose of review—Here we explore an emerging theme in the literature, which is the role of dendritic cells in the causation of fibrosis. To fully appreciate this pathway to disease, we also review the most recent literature regarding dendritic cell biology as it pertains to ocular surface tissues. Based on this information we propose a unifying hypothesis for how dendritic cells may cause conjunctival fibrosis in the allergy setting.

Recent Findings—Work in models of airway remodeling and liver fibrosis has pointed to a potentially central role for dendritic cells in the pathobiology of fibrosis. Indeed, these cells are recognized as the most potent antigen-presenting cells, and as such activate of T lymphocytes that are pro-fibrotic under certain conditions. However, recent findings suggest a more direct role for dendritic cells, which opens up the possibility that a similar pathway may be relevant in the causation of conjunctival fibrosis, particularly in allergic eye disease.

Summary—Conjunctival fibrosis is a serious clinical concern, and is associated with chronic inflammation of the ocular surface tissue, such as in allergic eye disease. Dendritic cells are required in mediating allergic disease by activating pathologic T lymphocytes. However, recent findings pointing to a central role for dendritic cell in fibrosis may mean that these cells could also be contributing directly to conjunctival fibrosis. If so, furthering our understanding of dendritic cells could lead to the identification of novel and more effective therapeutic strategies to treat this pathology.

Keywords

dendritic cell; fibrosis; allergy; conjunctival fibrosis; allergic conjunctivitis

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CONFLICTS OF INTEREST

DRS: Inventor on a patent involving CCR7 in ocular surface disease

SHD: Inventor on a patent involving fibrosis

1.0. Introduction

Fibrosis, or excessive scar tissue formation, is a common pathologic pathway to organ failure. Likewise for tissues of the ocular surface, fibrosis is a serious and even sight-threatening concern. Conjunctival fibrosis is associated with chronic inflammatory conditions, such as in allergic eye disease. Not necessarily in the mild or acute forms of seasonal or perennial allergic conjunctivitis (SAC, PAC), but rather in the more severe or chronic forms of vernal or atopic keratoconjunctivitis (AKC, VKC) [1]. Conjunctival fibrosis is also a concern in other chronic inflammatory diseases of the ocular surface, such as in mucous membrane pemphigoid [2–4]. In addition, although not reviewed here, corneal fibrosis is a clinically significant concern, and corneal involvement in allergic eye disease was recently described in mice [5].

Here we review an emerging theme, which is the role of dendritic cells (DC) in the causation of scarring and tissue remodeling [6–20], and relate this information to the pathobiology of conjunctival fibrosis in allergic eye disease. This topic is a fascinating one because DCs, which are present in most if not all tissues, have a primary function in antigen presentation/stimulation of T lymphocytes. Thus, it has come as unexpected that DCs can play a central role in fibroblast function/dysfunction. Indeed, as indirect players, DCs have been acknowledged in pathogenesis of fibrosis through activation of pro-fibrotic T cells, particularly in allergic diseases [19]. However, the possibility for a more direct contribution is also being considered, and reviewed herein.

2.0. Pathobiology of conjunctival fibrosis

Excessive subepithelial accumulation of collagen and other extracellular matrix (ECM) proteins that occurs in conjunctival fibrosis can be particularly progressive. Although increased ECM production is indeed part of normal healing, the pathologic dysregulation of this process results in a state where normal parenchyma is replaced with excessive and continual scarring that is permanent. In the conjunctiva, such responses cause forniceal shortening (Fig 1), and can progress to symblepharon and ankyloblepharon formation (Fig 1) – where excessive scar tissue has fused together the eyeball with the eyelids (Fig 1) [21].

Much of what is known about the underlying mechanisms in fibrosis is derived from studying how fibroblast activities differ in normal wound healing versus fibrosis settings. These cells are primarily responsible for homeostatic regulation of ECM. In a normal wound healing response, tissue resident fibroblasts migrate to the wound site, which is assisted through their expression of certain matrix metalloproteinases (MMP) [22–24] and cytoskeletal dynamics [22]. At the wound site, their contractile apparatus, collagen production and tissue remodeling capacities are enhanced [25–28], which ultimately leads to wound closure and apoptosis of these migrated fibroblasts. However in the case of fibrosis, where scarring is excessive, fibroblasts exhibit a pathologic phenotype that is maintained beyond the wound healing process. Fibroblasts explanted from fibrotic tissues, including the conjunctiva, produce abnormally heightened levels of collagen [4, 29–31], have altered contractile abilities, and proliferative profiles [30, 32, 33]. This pathologic fibroblast is commonly known as the myofibroblast (Fig 2) [34, 35]. These cells typically exhibit distinct

α -smooth muscle actin stress filaments, to increase contractility and strengthen ECM remodeling capacity (Fig 2), although their role remains unclear in conjunctival fibrosis.

While the precise mechanisms underpinning the progression of normal to pathologic fibroblast responses have not been fully elucidated, chronic inflammation is very commonly associated with this process. There is a large literature involving the pro-fibrotic role of macrophages (MFs), although recent studies suggest that certain MF populations can be reparative as well [19]. T lymphocytes are well documented as pro-fibrotic through their production of certain cytokines, such as IL-13 [18, 19, 36]. These lymphocytes also act indirectly via activation and recruitment of neutrophils or eosinophils that express pro-fibrotic cytokines, such as TNF- α , IL-1 β or TGF- β . Lastly, an emerging theme in the literature is the role of DCs in fibrosis [6–19], which we review in detail below.

3.0. Dendritic Cell Biology

There has been a recent explosion in our understanding of DCs, namely in the development and function of classical DCs (cDC), and it is important to appreciate this new information in order to accurately relate these cells potentially in fibrosis. cDCs are CD11c+ myeloid derived cells that exist constitutively in normal tissues. They are the foremost potent stimulators of T lymphocytes, and as such the main function of cDCs is antigen presentation. Antigen-laden DCs undergo the process of maturation in order to stimulate T cells, which involves the increase in their cell surface expression of class II major histocompatibility complex (MHC II) and costimulatory molecules (CD80, CD86, CD40), which is modulated in part by thrombospondin-1 [37]. Maturation is accompanied by upregulation of the chemokine receptor CCR7, enabling DC migration into terminal lymphatics and ultimately to the T cell rich paracortical areas of the regional lymph node [38]. Mature antigen-laden DCs then come in contact with and stimulate cognate receptor bearing naïve or memory T cells, and assist in T helper (T_H) cell differentiation (i.e. T_H1 , T_H2 and T_H17) [39].

Their highly specialized function in T cell stimulation means that it is of utmost importance to accurately identify cDCs. Features that do not distinguish cDCs *per se* are their morphology, i.e. the appearance of long dendritic processes. This is because not only are the shapes of DCs highly dynamic, other cells may have a dendritic appearance as well (e.g. epidermal $\gamma\delta$ T cells). Expression of CD11c in of itself is also not a distinguishable feature of DCs, as MFs express CD11c at significant levels (e.g. alveolar, splenic marginal zone, and thioglycolate induced MFs). In contrast, characteristics that positively distinguish cDCs are based on their unique developmental lineage (Fig 3). cDCs develop from precursor DCs (pre-DCs), which arise from the common DC progenitor lineage (CDP) in definitive hematopoiesis (Fig 3). This distinguishes cDCs from another type known as plasmacytoid DCs, which arise directly from CDPs as opposed to pre-DCs. This also distinguishes cDCs from so-called inflammatory/monocyte-derived DCs, which do not originate from the CDP lineage (Fig 3) [40–48].

3.1. Classical DC subsets and functions

It is now firmly established that cDCs are comprised of distinct and functionally specialized subsets [45]. Migratory (or non-lymphoid tissue) cDCs, i.e. those that migrate from peripheral tissues to lymph nodes, exist in two main subsets. These can be differentially identified in part by their positive expression of integrin CD11b versus CD103 in non-lymphoid tissues throughout the body (Fig 3), with some exception in the intestinal tract [46]. Both populations are derived from the CDPs, although there is some debate as to whether monocytes may also give rise to CD11b+ cDCs to a small extent. While the exact factors that govern differentiation of pre-DCs into CD11b+ versus CD103+ cDCs are unclear, some of the obligatory transcription factors (e.g. IRF4 and IRF8, respectively) in this process have been identified [40, 45, 47].

Our group has demonstrated the presence of *bona fide* CD11b+ and CD103+ cDCs in the conjunctiva (Fig 4), through cell surface expression, transcription factor profiles and developmental lineage analyses [38, 49]. Similar studies have not been conducted in the cornea; however, we have identified the presence of corneal stromal DCs that express the C-type lectin langerin and thus resemble CD103+ cDCs in the dermis [38, 50]. Also, the presence of CD11b expressing CD11c+ cells in the cornea is well documented, which may be akin to CD11b+ cDCs [51].

Regarding the functional specialization of these subsets, our group has shown that conjunctival CD11b+ cDCs play the dominant role in the pathobiology of allergic eye disease, and blocking the chemokine receptor CCR7 expressed on these cells inhibits this process [38, 52, 53] (Fig 5). Dry eye disease shows a similar level of improvement with therapeutic CCR7 blockade (Fig 5) [54]. The reasons for why CD103+ cDCs are not involved in this setting is not fully understood, although reports have converged that such cDCs are instead crucial in triggering immune responses against certain viruses [55]. Likewise, it is not fully understood why CD11b+ cDCs are dominant in allergic eye disease, although a common theme for such cDCs has emerged in other allergy models as well, such as in asthma [56, 57].

3.2. Langerhans Cells

Langerhans cells (LCs) are not cDCs. In fact, there is debate as to whether these cells should instead be classified as MFs, because like most tissue resident MFs (i.e. microglia, alveolar MFs, Kupffer cells, etc) they are derived from primitive (prenatal) not definitive (postnatal) hematopoiesis [42, 46]. LCs are relatively unique to the epidermis, where they were originally identified and are found in very large numbers. These cells possess Birbeck granules/organelles and express the C-type lectin langerin. It is inaccurate to assume that LCs exist universally in all other barrier tissues, or that all intraepithelial immune cells with a dendritic morphology are LCs. We have shown using (BAC transduced) human langerin mice [58, 59] that while LCs exist in the mouse cornea epithelium [50], these cells only make up a very small fraction of CD11c+ cells in the corneal epithelium [11]. Regarding their function, whereas epidermal LCs in skin immune responses has been well characterized [58, 59], their isolated role in the cornea is largely unknown.

4.0. Classical DCs in fibrosis pathobiology

As previously mentioned, DC biology is an emerging theme in the fibrosis literature. For example, studies have incorporated *in vitro* systems to help understand how mucosal and dermal fibroblasts influence DC function. Mouse models have also been incorporated, such as IL-1 β induced airway remodeling or common bile duct ligation in liver, which have implicated DCs in fibrosis pathobiology. Thus, whereas it has been recognized that cDCs contribute to fibrosis indirectly via activation of pro-fibrotic T_H cells, recent findings also point to the potential for a more direct means of triggering a pro-fibrotic state. Though much of the work on this topic is focused on the inflammatory/monocyte derived DCs, these early studies may provide the conceptual framework by which future investigations can now probe how cDCs may directly effect fibroblast function/dysfunction.

4.1. Classical DCs in T helper cell-mediated fibrosis

T_H2 cells are the primary pathogenic subset in allergic diseases, although T_H1 and T_H17 can also play appreciable roles in certain settings [39]. Interestingly, the T_H2 subset has emerged as a chief player in inducing and potentiating pro-fibrotic activity [18, 19, 36, 60], which explains the very high prevalence of fibrosis and tissue remodeling in chronic allergy. This proficiency of T_H2 is thought to be due to their expression of the highly pro-fibrotic cytokine, IL-13 [18, 19, 36, 60]. Fibroblasts are not only activated by direct ligation of this cytokine, but it also leads to augmented production of pro-fibrotic TGF- β [19]. In comparison, T_H17 cells, which produce IL-17, are recognized in fibrosis, albeit this is mostly as a secondary consequence of inflammatory tissue damage [18, 19, 36, 60]. Lastly, and in striking contrast, T_H1 responses are thought to be anti-fibrotic through IFN- γ production [18, 19, 36, 60, 61], for reasons not fully understood.

In conjunctival fibrosis, IL-13 has also received considerable attention as a pro-fibrotic factor. Leonardi *et al* showed that IL-13 (or IL-4) treated conjunctival fibroblasts had increased production of collagen and modified the equilibrium between MMP-1 and its inhibitor, TIMP-1 [61]. In a follow up study, Leonardi *et al* reported that IL-13 was highly expressed in tears of patients with SAC, AKC and VKC [62]; thus linking the role of IL-13 and aberrant functionality of conjunctival fibroblast in allergic eye disease. This concept has been further supported in studies from mucous membrane pemphigoid patients by *ex vivo* characterization of their primary conjunctival fibroblasts. These cells are functionally and morphologically abnormal [63], and characterized by a pro-fibrotic phenotype, e.g. increased collagen production [2–4]. Furthermore, these patients have increased ocular surface levels of IL-13 [3], and primary conjunctival fibroblasts exposed *ex vivo* with IL-13 display a pro-fibrotic profile [3]. It should be noted that other factors relevant in allergic ocular inflammation have been associated with fibrosis, including histamine and nerve growth factor [64, 65].

4.2. Classical DC-fibroblast crosstalk in co-culture systems

Since cDCs are positioned in close proximity to fibroblasts in the parenchyma, it is entirely conceivable that cross-regulation may occur between these two cell types and evidence exists to support this concept. Studies have revealed that fibroblasts have the potential to

induce a diverse set of effects on cDCs, although it should be noted that much of this work is based on *in vitro* studies using fibroblast-conditioned media or co-cultures with inflammatory/monocyte-derived DCs. The latter are used, instead of cDCs, because of the feasibility for obtaining large numbers of cells through differentiation of peripheral blood monocytes.

Using this system, several studies have reported an immunostimulatory role for fibroblasts. For example, Schirmer *et al* showed that IL-1 β /TNF- α stimulated human dermal fibroblasts produce prostaglandin E₂, which in turn promotes pro-inflammatory cytokine IL-23 production by inflammatory/monocyte-derived DCs and consequent T_H17 expansion in secondary cultures [66]. Previous *in vitro* studies have demonstrated that ICAM-1 and Thy-1 on dermal fibroblasts interact with β 2 integrins on inflammatory/monocyte-derived DCs [16]. This particular interaction not only promotes MMP-9 expression by inflammatory/monocyte-derived DCs to assist in their migration in matrigel assays, but also leads to maturation of these DCs [15]. Similar studies have not been conducted with conjunctival fibroblasts. However, TNF- α is increased in the conjunctivae of patients with allergy and mucous membrane pemphigoid, and exposure of primary fibroblasts to TNF- α results in a pro-fibrotic profile [2].

In contrast, a report by Segulier *et al* demonstrates that fibroblasts can be immunosuppressive [17]. Specifically, they showed that gingival fibroblasts inhibited differentiation of inflammatory/monocyte-derived DCs, and thereby reduced their consequent capacity to stimulate T cell proliferation in subsequent mixed lymphocyte reactions. Consistent with this finding, Berthier *et al* demonstrated that murine fibroblasts inhibited IL-12 production from, and maturation of, splenic cDCs [9]. Thus, whether inhibitory or stimulatory, fibroblasts may therefore have the potential to impact cDC function. However, whether the reverse also occurs, i.e. cDCs directly affecting fibroblast function, and if this type of regulation is relevant in conjunctival fibrosis is unknown.

4.3. Classical DC contribution in vivo fibrosis models

In vivo work to suggest crosstalk between cDCs and fibroblasts is not extensive, but there are some elegant studies that support this concept. For example, Kitamura *et al* showed in (allergen or IL- β induced) airway remodeling that cDC and inflammatory/monocyte-derived DC recruitment to the lung is dependent on fibroblast α v β 8-mediated activation of TGF- β [20]. Whether, through this mechanism, fibroblasts directly recruit these cells or if recruitment is an ancillary effect of inflammation, requires further investigation. Nonetheless, this process may be relevant in conjunctival fibrosis, as a role for TGF- β has been well documented. In another report, Connolly *et al* showed in a mouse model of liver fibrosis that CD11c+ expressing cells, via TNF- α , govern hepatic inflammation [10]. Interestingly, increased levels and contribution of TNF- α has also been shown in conjunctival fibrosis [2]. It should be noted that CD11c+ cells shown by Connolly *et al* likely represent the inflammatory/monocyte-derived type rather than *bona fide* cDCs. Furthermore, whether cDCs directly contribute to fibrosis, rather than indirectly through tissue inflammation remains to be determined.

Other studies point away from cDCs in the causation of fibrosis, and even goes as far as to implicate a reparative function. A recent study by Pradere *et al*, using a model of common bile duct ligation, showed that hepatic MFs contribute to liver fibrosis, as opposed to cDCs [13]. Authors demonstrate that hepatic MFs achieve this result in an IL-1 and TNF mediated manner. In another study by Jiao *et al*, cDCs were elegantly shown to actually promote the regression of liver fibrosis in mice [11]. Authors demonstrated that resolution was partially dependent on DC expression of matrix metalloproteinase (MMP)-9.

5.0. Conclusions and Future Directions

The following unifying hypothesis is based on the available literature, and can be proposed for how cDCs may cause conjunctival fibrosis in allergic eye disease. This result may occur in multiple ways that are not mutually exclusive. One manner might be through CD11b+ cDC expression of pro-inflammatory TNF- α (Fig 6 a), which contributes to inflammation (Fig 6 b) and fibroblast activation (Fig 6 c) [10]. Separately, CD11b+ cDCs may activate fibroblasts indirectly through stimulation of T_H2 [38, 49, 52, 53], which produce IL-13 and TGF- β . This also leads to recruitment of eosinophils and consequent inflammation (Fig 6 a) that furthers tissue damage [19, 36]. Activated fibroblasts in turn trigger or contribute to a positive feedback loop by contributing to inflammation (Fig 6 c) [20], which leads to recruitment of inflammatory/monocyte-derived DCs (Fig 6 d) [10, 20], or additional CD11b + cDCs [10, 20] to the allergic conjunctiva (Fig 6 d). Perpetuation of this positive feedback in chronic forms of ocular surface inflammation, such as in VKC and AKC, may ultimately lead to fibrosis.

The question for future studies is whether cDCs can directly activate fibroblasts in the conjunctiva or elsewhere in a manner that ultimately leads to fibrosis. Indeed, cDCs can contribute to inflammation, which may lead to fibrosis secondarily. Also, cDCs can produce pro-fibrotic factors, such as TGF- β ; however a wide array of cells make this cytokine and so the precise role of cDC derived TGF-B is unclear. Thus, the question of whether cDCs directly cause fibrosis and whether this is relevant in allergic eye disease remains unresolved. The importance of addressing this question is not solely of academic merit, but may also be very useful in developing novel therapeutic strategies in the prevention or treatment of conjunctival fibrosis.

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KEY POINTS

- Fibrosis is a common pathologic pathway to organ failure and is likewise a serious and sight threatening concern in allergic eye disease.
- Whereas classical dendritic cells in mice include CD11b+ and CD103+ subsets, which have functional equivalents in humans, it is the CD11b+ subset that plays the dominant role in allergic eye disease immunopathogenesis.
- Recent work suggests that classical dendritic cells can play a central role in fibrosis pathobiology, which means that these cells may contribute to conjunctival fibrosis in allergic eye disease.

Figure 1. Clinical presentation of patients with conjunctival fibrosis

Patients presenting with conjunctival fibrosis may begin to exhibit subepithelial fibrosis where there is a sheet of scarring maintained underneath the epithelium. This can further develop into fornix shortening where the area between the eyelid and globe becomes shallow due to building conjunctival scar tissue. Symblepharon formation then develops when bands of scar tissue begin to physically pull the eyelid towards to the globe. Ankyloblepharon is end stage conjunctival fibrosis and this is where the globe and eyelid have fused together. Adapted from [21]

Figure 2. Fibroblast activation and myofibroblast differentiation

In the presence of pro-fibrotic factors such as TGF-beta, and mechanical tension, a fibroblast will enhance its expression of alpha smooth muscle actin and subsequently assemble alpha smooth muscle actin stress fibers. These alpha smooth muscle actin stress fibers are the hallmark of a differentiated myofibroblast.

Bone Marrow

Figure 3. Ontogeny of DCs

cDCs develop postnatally through definitive hematopoiesis in the bone marrow, whereas LCs are seeded from embryonic precursors via primitive hematopoiesis. cDCs arise from pre-DCs, which is in contrast to plasmacytoid DCs that arise from CDPs. Inflammatory/monocyte-derived DCs also have a different lineage, as these cells develop from monocytes. Adapted from [38]

Figure 4. The presence of CD11b+ and CD103+ cDCs in mice, which have functional equivalents in humans, is conserved in most tissues

Flow cytometry can be used to identify cDCs in the conjunctiva and elsewhere, such as the lung. To reveal CD11b+ and CD103+ cDCs, cells (live, singlets) must be gated on CD11c+ and I-A/E+ (i.e. MHC II), and then on autofluorescent negative (cells to remove macrophages from analyses). Adapted from [49]

(a)

(b)

Isotype Ab

anti-CCR7 Ab

Figure 5. Robust therapeutic efficacy of topical CCR7 blockade in dry eye disease and allergic eye disease

(a) Allergic eye disease in mice is induced by systemic immunization with allergen and subsequent chronic exposures of allergen. CCR7 blocking antibody was applied topically. Adapted from [53] (b) Dry eye disease is induced in mice via hyper-desiccating stress. CCR7 blocking antibody was applied topically. Adapted from [54].

Figure 6. Unifying hypothesis for the role/s of cDCs in the pathogenesis of conjunctival fibrosis in allergic eye disease

(a) CD11b+ cDCs can express TNF- α , and activate IL-13 and TGF- β producing T_H2 cells. (b, c) These activities contribute to inflammation and promote fibroblast activation/myofibroblast differentiation. (d) In turn activated fibroblasts promote a positive feedback loop by also contributing to inflammation, which leads to recruitment of inflammatory/monocyte-derived DCs, or additional CD11b+ cDCs to the allergic conjunctiva. Perpetuation of this positive feedback loop in chronic inflammation may ultimately lead to

fibrosis and significant tissue remodeling. Whether cDCs can directly contribute to fibroblast activation and consequent fibrosis is unknown.

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