Review article

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FIBROBLASTS: THE UNKNOWN SENTINELS ELICITING IMMUNE RESPONSES AGAINST MICROORGANISMS

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Fibroblasts are present in all tissues but predominantly in connective tissues. Some of their functions include contractility, locomotion, collagen and elastin fiber production, and the regulation and degradation of the extracellular matrix. Also, fibroblasts act as sentinels to produce inflammatory mediators in response to several microorganisms. There is evidence that fibroblasts can synthesize toll-like receptors (TLRs), antimicrobial peptides, proinflammatory cytokines, chemokines, and growth factors, which are important molecules involved in innate immune response against microorganisms. Fibroblasts can express TLRs (TLR-1 to TLR-10) to sense microbial components or microorganisms. They can synthesize antimicrobial peptides, such as LL-37, defensins hBD-1, and hBD-2, molecules that perform antimicrobial activity. Also, they can produce proinflammatory cytokines, such as TNFα, INFγ, IL-6, IL-12p70, and IL-10; other chemokines, such as CCL1, CCL2, CCL5, CXCL1, CXCL8, CXCL10, and CX3CL1; and the growth factors granulocyte/macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) to induce and recruit inflammatory cells. According to their immunological attributes, we can conclude that fibroblasts are sentinel cells that recognize pathogens, induce the recruitment of inflammatory cells via cytokines and growth factors, and release antimicrobial peptides, complying with the characteristics of real sentinels.

Keywords: fibroblasts, sentinel, microorganisms, cytokines, antimicrobial peptides

Introduction

Physical barriers are the first line of defense to prevent the entrance or establishment of microorganisms [1], and connective tissue participates actively in immune response [2]. The fibroblast is the principal cell that synthesizes connective tissue, and it is considered the main workhorse of this tissue [3].

Fibroblasts are present in all tissues but predominantly in connective tissues. Their origin is mesenchymal, and, depending on their localization, they have multiple morphologies. For example, they may be flattened, elongated, or of a certain spindle-shape, they may contain either one or two oval nuclei, and they are smoothly contoured with a concave and bordered projection. Some of their functions include contractility, locomotion, collagen and elastin fiber production, and the regulation and degradation of the extracellular matrix [4, 5].

Fibroblasts play an important role in wound healing and structural support, and they act as sentinels in produc-

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ing inflammatory mediators (cytokines, chemokines, and growth factors) as well as acting in response to infections by several microorganisms [6–8].

The sentinel concept is defined in this way: "A subject who watches something and occupies a place and asks for entry passwords." Given this definition, we think that fibroblasts have an immune repertoire in responding to different microbial agents. In this review, we will cover the immunologic components of fibroblasts that are expressed in an infection process. There is evidence that fibroblasts can synthesize toll-like receptors (TLRs), antimicrobial peptides, proinflammatory cytokines and chemokines, and growth factors, which are important molecules involved in an innate immune response against microorganisms. Their role is very important in expanding immune response and solving microbial infections. Previously, their role was believed to be related to functions connected to fibrosis and the repair of damage. However, other studies suggest that fibroblasts should be considered sentinels, and we concur with this and further believe that their role is as sentinels against microbial pathogens.

Can fibroblasts express TLRs against microorganisms or microbial components?

The answer is yes.

All good sentinels must have components by which they can recognize the enemy agents; in this case, the enemies (microorganisms or microbial components) are recognized by TLRs: These sentinel molecules recognize pathogens and ask them, "Where is your ticket to enter this place?"

We know that the innate immune system is the first line of response to pathogens, and this response generally includes three events: microbial recognition, activation of signaling pathways, and activation of an effector mechanism. The recognition of pathogens is mediated by TLRs, which are membrane proteins that recognize specific pathogens associated molecular patterns (PAMPs). TLRs have an amino terminal leucine rich repeat (LRR) domain and an intracellular carboxyl terminal domain that contains a conserved region known as the toll/intercellular receptor (TIR). The TLR family comprises 10 TLRs (TLR1 to TLR10 have been reported thus far) [9, 10].

The recognition of PAMPs by TLRs can occur at the cell surface (TLR1, TLR2, TLR4, TLR5, and TLR6) and in intracellular vesicles (TLR3, TLR7, TLR8, and TLR9). TLRs play an important role in recognizing microbial components from bacteria, fungi, parasites, and viruses [11].

TRL1, TLR2, and TLR4 recognize bacterial cellular walls, and TLR2 forms heterodimer complexes with TLR1 or TLR6. TLR3 recognizes polyinosinic–polycytidylic acid (poly I:C) and double-stranded viral ribonucleic acid (RNA). The flagellin of gram-negative bacteria can be recognized by TLR5 and TLR7, while TLR8 recognizes single-stranded viral RNA and TLR9 identifies viral and bacterial deoxyribonucleic acid (DNA) unmethylated CpG motifs [9, 10, 12], and expression of TLR10 has been induced by exposure to *Helicobacter pylori* [13].

Yao and colleagues demonstrated the expression of 10 different TLRs in skin fibroblasts and also showed that fibroblasts are functionally active (TLR1 to TLR9, but not TLR10). The authors further showed the importance of fibroblasts in sensing PAMPs and in synthesizing the cytokines and antimicrobial peptides involved in an immune response [14].

A gram-positive cellular wall possesses many layers including peptidoglycan and lipoteichoic and teichoic acids [1]; these PAMPs can be recognized by corneal fibroblast TLRs. Peptidoglycan (PGN; fsa = from *Staphylococcus aureus*) induces the specific expression of TLR1, TLR2, and TLR6 for these PAMPs, but also the expression of TLR5, TLR7, or TLR8, which are not specific for these PAMPs, while stimulation with lipoteichoic acid (LTA; fsa) induces TLR5 expression and muramyl dipeptide (MDP; fsa) induces TLR9 expression [15].

On the other hand, gram-negative bacteria possess a slim PGN layer, while in the outer membrane there is a lipopolysaccharide (LPS) formed of lipid A and O-linked polysaccharide [1, 16]. There are components of gram-negative bacteria that can induce TLR corneal and gingival fibroblast expression, such as an LPS from *Pseudomonas aeruginosa,* the tetra-acylated lipid A LPS from *Porphyromonas gingivalis,* and the LPS from *Escherichia coli* which also induces TLR4 expression [16–18].

The synthetic CpG oligodeoxynucleotide (CpG ODN) is another component that mimics the response to bacterial DNA. CpG ODN is a potent activator of immune response [19]; its CpG ODN induces TLR9 response on synovial fibroblasts [20].

It has been demonstrated that fibroblasts express TLR4 in the presence of the hyphae or conidia of *Fusarium solu* [21]. This expression has been observed in other diseases where corneal cells expressed TLR2 and TLR4 in the presence of *Aspergillus fumigatus* [22].

Fibroblasts can recognize viral molecules. TLR3 is implicated in the recognition of viral molecules such as double-stranded RNA (dsRNA) [9]. This behavior was observed in myofibroblasts stimulated with poly I:C that mimic a viral infection [23]. Epstein Barr virus is a γ-herpes virus involved in systemic sclerosis (SSc) or scleroderma. This virus induces TLR3 expression in fibroblasts [24]. These data indicate the ability of fibroblasts to recognize PAMPs through the TLRs of different microorganisms and to carry out a synthesis of antimicrobial peptides, proinflammatory cytokines, and chemokines, as well as growth factors, to contain the pathogens *(Fig. 1).*

The sentinel fibroblasts said, "We are difficult fellows, and we detect enemies. Their names are pathogens, and they cannot win with us."

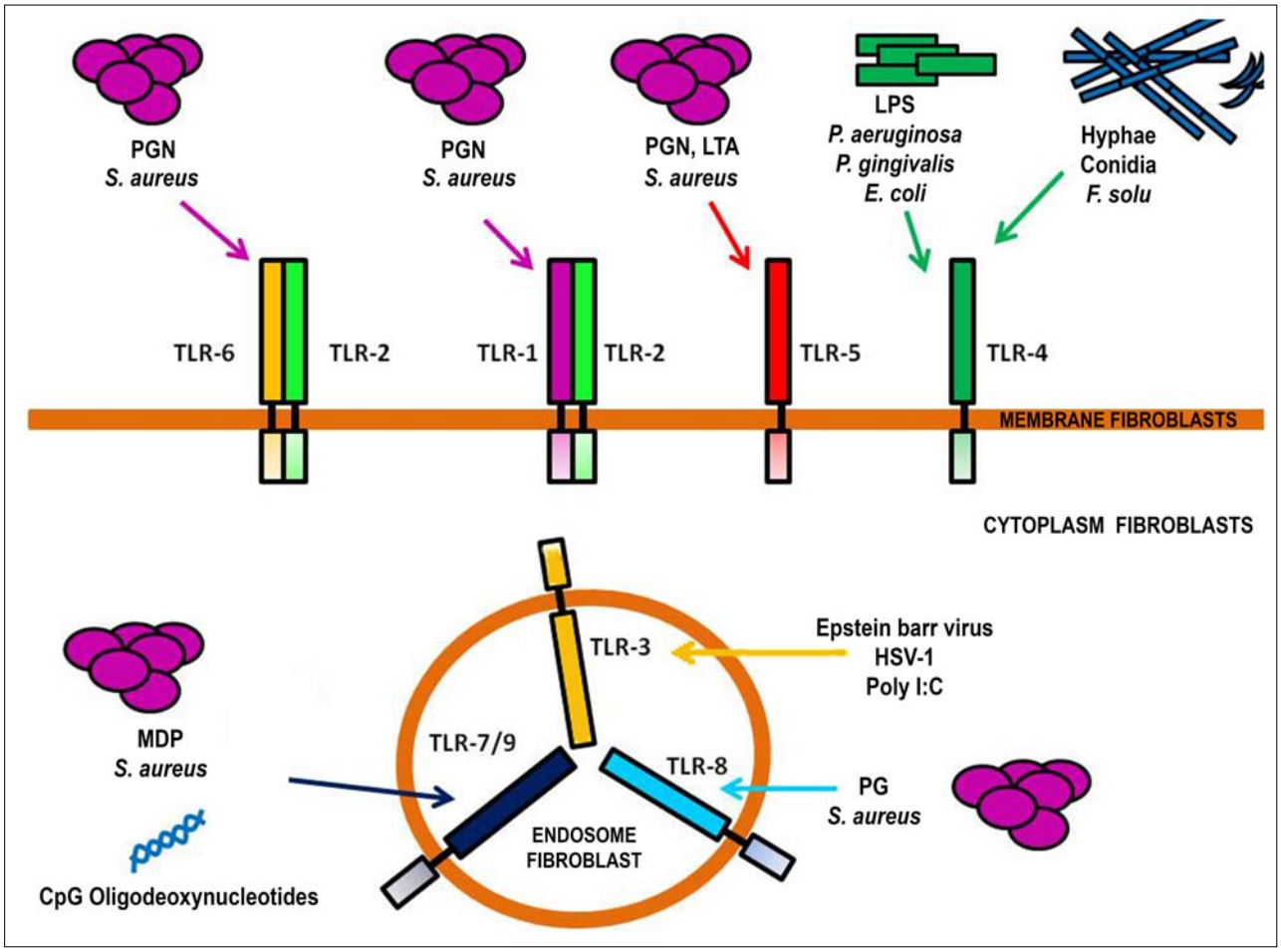

Fig. 1. Toll-like receptor expressed in fibroblasts against microbial ligands. *Staphylococcus aureus* components can activate TLRs, and PGN activates TLR1, TLR2, TLR3, TLR5, and TLR8. MDP from *S. aureus* increases the TLR9 expression. LPS from *P. aeruginosa,* tetra-, and penta-acylated lipid A of LPS from *P. gingivalis* and *Fusarium solu* conidia or filaments induce TLR4 expression. Epstein Barr virus, HSV-1, and poly I:C induce TLR3 expression in fibroblasts

What about antimicrobial peptide synthesis by fibroblasts in the presence of microorganisms or microbial components?

Good sentinels have different weapons; in this case, the fibroblasts have important molecules used as antimicrobial peptides when they are stimulated with pathogens or when pathogen components produce antimicrobial peptides. The fibroblast sentinels demand, "Where are you going, bad boy (pathogen)?" Then they shoot antimicrobial peptide-reaching pathogens.

Antimicrobial peptides are important components of the host innate immune response. They perform activity against gram-positive and gram-negative bacteria, fungi, and viruses. This family includes α-defensins and β-defensins (hBD1 to hBD4), adrenomedullin, histatins, and cathelicidins (LL-37) [25].

Results from osteoblast cultures revealed that LL-37 is capable of eliminating extra- and intracellular *S. aureus* and showing more antimicrobial activity than conventional antibiotics, such as doxycycline and cefazoline. However, the LL-37 concentrations and the susceptibility of *E. coli* to LL-37 increase the probability of developing urinary tract infections (UTI) [26, 27].

β-Defensin 3 (hBD-3) is able to suppress the biofilm formation of *S. aureus* [28]. Common ocular pathogens (*P. aeruginosa, S. aureus,* and *S. epidermidis*) challenged with hBD-3 decreased their viability in a dose-dependent fashion [29].

Fibroblasts produce defensins and cathelicidins when challenged by microorganisms. In several studies, limbo-corneal fibroblasts produced β-defensin hBD-1 and cathelicidin LL-37 when stimulated with mycobacteria (*Mycobacterium tuberculosis, M. smegmatis,* and *M. abscessus*); on the other hand, gingival fibroblasts secreted hBD-2 in the presence of intracellular bacteria such as *Chlamydia* spp., while corneal fibroblasts expressed DEFA-3, which is an α-defensin, when they were stimulated with PGN from *S. aureus (Table 1)* [30–32].

This information indicates that the fibroblasts secrete molecules important to the innate immune response with respect to antimicrobial activity. The fibroblasts said, "We have and effective weapon against the pathogens. We are amazing cells!"

Table 1. Immunological molecules synthesized when the fibroblasts were stimulated with different microbial components or microorganisms

Fibroblast strain	Microbial component or microorganisms	Cytokine/growth factor/antimicrobial peptide
HGF [16]	LPS tetra acylated peptide A	GM-CSF, CXCL10, G-CSF, IL-6, IL-8, and CCL2
HDF [17]	Flagellin	CXCL8 (IL-8)
CF [18]	*P. aeruginosa* LPS	MMP-9, IL-2, IL-8, IL-10, IL-12p70, GM-CSF, INFγ, TNFα, IL-6, MCP-1, and MIP-1β (CCL4)
SFHTI [21]	Hyphae and conidia *Fusarium solu*	IL-10, IL-1β
LCM [23]	Poly I:C	CCL1, CCLC, CCL5, CXCL1, CXCL8 (IL-8), CXCL10, G-SCF, IL-6
LCF [30]	Mycobacteria	hBD1 and LL37
EF [38]	Biofilm and planktonic supernant	INFγ, IL-6, IL-8, VEGF, TGF-β1, EGF HB, and MMP-3
OCF [39]	*C. albicans*	CX3CL, IL-6, IL-8
HGF [40]	Chlamydia sp	IL-6, IL-8, hBD2

HGF = human gingival fibroblasts; HDF = human dermal fibroblasts; CF = corneal fibroblasts; SFHTI = stromal fibroblasts human telomerase immortalized; LCM = limbo-corneal myofibroblasts; LCF = limbo-corneal fibroblasts; EP = epidermal fibroblasts; OCF = oral cavity fibroblasts

What about the synthesis of cytokines expressed by fibroblasts against present microorganisms?

All the sentinels have devices by which to communicate with their crew partners and to try to stop and eliminate intruders (pathogens). The sentinel fibroblasts said to their crew partners, "We think that we saw a nasty pathogen", and they released the alarm (proinflammatory cytokines and chemokines) to notify the remaining cells.

Cytokines are proteins secreted by the cells of the innate or adaptive immune response; these proteins can be secreted in the presence of microorganisms and can induce the activation of different effector cells. Cytokines are known as interleukins (ILs) and are secreted by different immune cell strains [33].

The interferon gamma (INFγ) is an important cytokine directed against both intracellular and extracellular pulmonary pathogens. INFγ increases the cytotoxic and phagocytic activity of macrophages, restricting the growth of infected cells [34, 35]. IL-6 is an inflammatory cytokine and is synthesized and activated in an acute immune response when infection occurs [36]. IL-12p70 is synthesized by dendritic cells when they are stimulated with major membrane protein (MMP)-II, in addition to producing tumor necrosis factor alpha (TNFα) [37].

Skin fibroblasts can synthesize proinflammatory cytokines such as INFγ, IL-6, and IL-8 in response to conditioned biofilm and planktonic cultures of *S. aureus* cells [38]. Corneal fibroblasts stimulated with LPS from *P. aeruginosa* synthesize IL-6, IL-2, IL-10, IL-12p70, and INF-γ [39]. Human gingival fibroblasts express IL-6 and IL-8 in the presence of LPS tetra-acylated peptide A from *P. gingivalis* [23]. The same effect occurs when the oral fibroblast cavity is exposed to *C. albicans* and *Chlamydia* spp. [31, 39]. Finally, limbo-corneal myofibroblasts synthesize IL-6 in the presence of poly I:C [23].

TNFα is a cytokine with inflammatory and autoimmune activity which provides different responses to tissue damage, fever, tumor necrosis, proliferation, differentiation, and apoptosis, as well as a number of other conditions. Monocytes, macrophages, natural killer cells, lymphocytes, mast cells, Paneth cells, mesenchymal intestine cells, and keratocytes, among others, can produce TNFα. In bacterial infections, TNFα is released by intestinal epithelial cells. The exposure of intestinal epithelial cells to LPS induces the production of TNFα [40]. In corneal fibroblasts, it has been found that stimulation with LPS from *P. aeruginosa* induces TNFα expression [18].

Chemokines are cytokines that function to regulate monocyte and leucocyte trafficking and induce migration to peripheral sites of the pathogen challenge. Chemokines are subdivided as CC, CCX, and CX3C [41, 42].

In malarial infections, there are the synthesis of proinflammatory cytokines and the recruitment of monocytes and macrophages; there are also expressions of chemokines such as CCL2, CCL3, CCL4, CXCL8 (IL-8), CXCL9, CXCL13, and CXCL16 in the placentae of women with malaria [41]. The chemokine CXCL8 is primarily produced by fibroblasts and other cells in response to periodontal bacteria and bacterial components [42]. The expression of CXCL10 increases in airway epithelial cells when the cells are stimulated with dsRNA and INFγ [43]. CXCL10, CXCL9, CCL2, CCL3, and CCL5 are involved in the cellular migration of natural killer cells (NK), macrophages, T cells, neutrophils, and plasmacytoid dendritic cells in a model of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) [44].

Dominguez et al. demonstrated the expression of chemokines, such as CCL1, CCL2, CCL5, CXCL1, CXCL8 (IL-8), and CXCL10, on limbo-corneal myofibroblasts with the stimulus of poly I:C [23]. Herath et al. showed

the expression of CCL2 and CX3CL1 by gingival fibroblasts [16]. Previous data had suggested the capacity of fibroblasts to induce chemokines when there was a microbial infection and to cause the recruitment of inflammatory cells *(Table 1)*.

The sentinel fibroblasts said that their communication method with the gremmie members was the best. "We do not need social networks to organize the response against pathogenic agents."

Can fibroblasts synthesize growth factors in the presence of microorganisms or microbial components?

The sentinels have other instruments with which to defend against intruders and to stimulate cell proliferation as growth factors. The sentinel fibroblasts release another alarm: "Hey partners, this is the second warning. Come with us! The intruder will not escape."

Vascular endothelial growth factor (VEGF-A) is a factor involved in inflammatory neovascularization, and it is able to recruitment monocytes/macrophages [45]. VEGF-A increases in *C. albicans* keratitis; this growth factor was present in the epithelium and stroma of infected corneas in a murine model of fungal keratitis [46]. Fibroblasts are also important stromal cells. Kirker and colleagues found VEGF expression in epidermal fibroblasts challenged with supernatants from planktonic cells of *S. aureus* [38]. This information indicates the ability of the fibroblasts to synthesize VEGF, to promote the recruitment of cells, such as monocytes and macrophages, and to induce inflammatory neovascularization.

The growth factors granulocyte/macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) play roles in immune responses, survival, cancer pathogenesis, proliferation of macrophages, erythrocytes, eosinophils, megakaryocytes, and multipotent progenitors. The expression of GM-CSF is associated with inflammatory diseases, such as rheumatoid arthritis and inflammatory renal disease. GM-CSF induces proinflammatory cytokines in response to exposure to LPS in mice. The production of G-CSF is induced by TLRs and ligands such as LPS. The G-CSF induces the recruitment of neutrophils to sites of inflammation [47, 48].

The production of growth factors by fibroblasts has also been reported [47]. According to Herath's study, *P. gingivalis* induced the expression of GM-CSF in human gingival fibroblasts [16], and Lu et al. [49] observed the synthesis of GMC-CSF in corneal fibroblasts when stimulated by *P. aeruginosa* LPS, while Dominguez-Lopez reported poly I:C-induced G-CSF expression on limbo-corneal myofibroblasts [23]. These reports permit us to conclude that the fibroblast produces GM-CSF upon bacterial stimulation and G-CSF in a viral infection. These growth factors indicate that the fibroblasts recruit inflammatory cells in response to microorganisms and induce cellular proliferation *(Table 1)*.

Conclusions

The innate immune response comprises different mechanisms to defend the host against pathogenic agents, for example, in the case of the physical barriers in the skin or ocular surfaces where the barriers are constituted by layers that have specialized cells with certain functions. The fibroblasts are stromal cells that play an important role in establishing a response against microorganisms. We consider that fibroblasts are stromal sentinel cells that possess "weapons" as TLR's to recognize microbial agents. When the pathogens are recognized, the fibroblasts begin to synthesize cytokines and growth factors to expand the immune responses and finally secrete antimicrobial peptides as "mortal weapons" to prevent or eliminate the establishment of the invading enemies.

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Conflict of interests

The authors declare no conflict of interest.

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