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DOI:
10.1038/s41577-021-00540-z

Document Version
Peer reviewed version

Citation for published version (Harvard):
Davidson, S, Coles, M, Thomas, T, Kollias, G, Ludewig, B, Turley, S, Brenner, M & Buckley, CD 2021, 'Fibroblasts as immune regulators in infection, inflammation and cancer', *Nature Reviews Immunology*, vol. 21, no. 11, pp. 704-717. https://doi.org/10.1038/s41577-021-00540-z

Fibroblasts as immune regulators in infection, inflammation and cancer

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Abstract

In chronic infection, inflammation and cancer, the tissue microenvironment controls how local immune cells behave, with tissue-resident fibroblasts emerging as a key cell type in regulating activation or suppression of an immune response. Fibroblasts are heterogeneous cells, encompassing functionally distinct populations, the phenotypes of which vary according to their tissue of origin and type of inciting pathology. Their immunological properties are also diverse, ranging from the maintenance of a potent inflammatory environment in chronic inflammation, to promoting immunosuppression in malignancy and encapsulating and incarcerating infectious agents within tissues. In this review we compare the mechanisms by which fibroblasts control local immune responses, as well as the factors regulating their inflammatory and suppressive profiles, in different tissues and pathological settings. This cross-disease perspective highlights the importance of tissue context, in

determining fibroblast-immune cell interactions, as well as potential therapeutic avenues to exploit this knowledge for the benefit of patients with chronic infection, inflammation and cancer.

1.0 Introduction

Early histological studies in the late 19th century identified fibroblasts by their distinct spindle shaped morphology, a characteristic that delineates them from other structural cells, such as epithelium and endothelium [1,2]. While fibroblasts were classically thought of as "immune neutral" cells, whose primary function is the construction and remodelling of the extracellular matrix (ECM)[3], it is now clear that fibroblasts play a multifaceted role in health and disease. In particular, fibroblasts have emerged as key immune-sentinel cells, activating and modulating immune response upon the detection of pathological stimuli. Like myeloid cells, fibroblasts can detect damage associated molecular patterns (DAMPS) and pathogen associated molecular patterns (PAMPs), activating pro-inflammatory signalling pathways to aid leucocyte recruitment and regulate their activity [4–7]. . As such, these cells are now acknowledged as a 'non-classical' branch of the innate immune system.

Although there are common mechanisms that fibroblasts use to regulate tissue immunity across diseases, others are unique to a single pathology or anatomical location. These properties are shaped by their local tissue environment, as well as specific inciting pathogenic signals. Furthermore, new single cell technologies have revealed that the fibroblast compartment encompasses functionally distinct subpopulations in homeostasis and disease. The identity and function of these populations in health also contribute to their ensuing pathogenic phenotype. Understanding how these elements combine to shape fibroblast function is crucial for the development of therapeutics. Identifying factors regulating fibroblast phenotype in one disease, may be harnessed or repurposed to treat others. For instance, induction of immunosuppressive mechanisms, utilised by fibroblasts in cancer, may aid resolution of chronic immune mediated inflammatory disease. By examining our current understanding of fibroblastimmune interactions- in infection, inflammation and cancer, this review will discuss these themes, aiming to draw parallels and differences across tissue and diseases.

2.0 Fibroblast subsets in health and disease

Despite playing an integral role in tissue homeostasis and pathological processes, our understanding of fibroblast function has been hampered by their intrinsic heterogeneity and a lack of robust markers. While markers such as platelet derived growth factor receptors (PDGFR) α and β, podoplanin (PDPN), Thy1 (CD90) and alpha smooth muscle actin (αSMA) are commonly used to distinguish fibroblasts, many of these markers are neither uniformly nor uniquely expressed by these cells. Indeed, fibroblasts are still identified by the absence of molecules associated with other lineages, such as endothelial (CD31), epithelial (EPCAM) or hematopoietic (CD45) cells. However, revolutions in minimally invasive surgery, including ultrasound mediated biopsy, combined with the advent of technologies such as single cell RNA sequencing (scRNAseq), have transformed our ability to catalogue and assign potential functional properties to distinct fibroblast subsets in human pathology. Furthermore, coordinated international efforts from consortia such as the Human Cell Atlas, ImmGen and the Accelerating Medicines Partnership, have significantly enhanced our understanding of the cellular composition of multiple tissues and biological systems. This approach has begun to unlock the cellular basis of disease, revealing an exciting variety of fibroblast subsets with non-overlapping functional roles. These populations and their unique characteristics will now be reviewed across infection, inflammation and cancer. We will not review the impact of these technologies on fibroblasts responsible for fibrosis as this has been recently covered [8].

2.1 Fibroblast heterogeneity in health As early as the 1960s, disparity in functional properties, such as proliferation and secreted factors, was noted between cultured fibroblasts from different tissues [9]. However, such diversity also exists within a single anatomical location. For example, the transcriptional signatures of the dermal mesenchyme vary across the limbs, diverging from the torso towards the fingers or toes [10,11]. Similarly, fibroblasts isolated from different synovial joints display unique expression profiles. In both cases, this feature was retained *in vitro*, suggesting that fibroblasts are imprinted with positional identity. Such imprinting occurs during development and is maintained in the postnatal period by epigenetic regulation of HOX genes [12]. These location specific transcriptional programs are likely to reflect the individual functional requirements of the surrounding tissue. For example, fibroblasts residing in epithelial tissue, such as the gut, possess specialised features to support epithelial cells, in order to maintain barrier integrity

and function [13]. Conversely, in the absence of any epithelium, synovial fibroblasts form a distinct lining layer with resident macrophages which helps maintain synovial health and joint lubrication [14]. This concept is supported by a recent scRNAseq study, which demonstrated that fibroblast transcriptional programmes bear more similarities to other structural cells within their organ ecosystem, than fibroblasts from other locations[15].

Furthermore, within a single anatomical location, specialised fibroblast subsets maintain discreet functional sub-compartments. The spatial distribution of fibroblasts in the gut is tailored to the needs of the local tissue environment. For example, SOX6+ POSTN+ fibroblasts, are positioned near the epithelium and display a transcriptional signature indicative of a a role in epithelial differentiation and proliferation [13,16,17]. Moreover, expression of WNT components varies according to spatial location. Specifically, fibroblasts expressing WNT5A/5B reside closer to the villus, whilst those located in the lamina propria express WNT2B [13]. Similarly, in the lung, Axin2+ PDGFRα+ fibroblasts reside in the alveolar niche. In organoid cultures, this fibroblast population enhanced self-renewal and differentiation of alveolar stem cells, compared to other subsets. This is mediated by production of FGF7, IL6 and the BMP inhibitor, Grem2 [18].

Skin and hepatic fibroblasts also display zonal demarcation. In the dermis, fibroblasts residing in the upper papillary region are spindle shaped, proliferate and help maintain the epidermal structure [19–21], while those of the lower reticular dermis are flatter, less proliferative and display increased expression of αSMA [19]. Concurrently, NGFR+ hepatic stellate cells (HSCs), a population of liver fibroblastic stromal cells, reside close to the portal vein, whereas those surrounding the central vein express Adamdtsl2+ [22]. Interestingly, gene cassettes associated with HSCs from different zones, mirror those of surrounding endothelial cells. This supports the hypothesis that tissue specific properties are imprinted across structural cell types and reflect local organisation.

2.2 Fibroblast heterogeneity in disease. In disease, homeostatic populations expand and gain pathological features that help drive disease progression and persistence. Similar pathological stimuli elicit common functional programmes across organs. For example, fibrosis of the lung and liver induces a fibrotic ‘myofibroblast’ transcriptional program, in discrete local populations.Axin2+ PDGFRα- pulmonary fibroblasts, a population distinct from

the Axin2+ PDGFRα+ subset of the alveolar niche [18], and central vein HSCs, drive fibrotic disease in the lung and liver respectively [22]. A similar phenomenon occurs upon viral stimulation, which activates an immune programme in fibroblasts in kidney, lung, skin and liver [15]. However, immune signatures were unique to each organ, indicating pathogenic phenotypes also reflect the surrounding tissue niche.

Furthermore, within individual tissues and disease, distinct fibroblast subsets can mediate different pathogenic responses. In the cancer field, this concept was pioneered by Ohlund *et al*, who uncovered functionally distinct populations of cancer associated fibroblasts (CAFs) [23]. Enriched in the tumour microenvironment, CAFs are fibroblastic cells that aid malignant growth and development via an array of pathogenic properties. These tumour-supporting functions distinguish these cells from their normal counterparts and include promoting angiogenesis, developing a fibrotic matrix, mediating metastasis and modulating immune infiltrates [24,25]. In pancreatic adenocarcinoma (PDAC), Ohlund et al, identified t αSMA+ myofibroblasts juxtaposed with a discrete IL6 expressing CAFs, termed myCAFs and iCAFs respectively. A unique *in vitro* system further revealed myCAFs display a matrix-remodelling gene expression profile, while iCAFs express cytokines and chemokines indicative of immune-cross talk. [23]. Following this study, the landscape of the mesenchymal compartment has been extensively profiled across a number of murine tumour models and human malignancies. These include breast, pancreatic, melanoma, lung, glioblastoma and head and neck [26–32]. One of the first studies investigating the functional properties of distinct fibroblast populations in cancer was performed by Costa *et al,* in human breast tumours [27]. Using flow cytometry, the authors identified four populations based on their expression of CD29, FSP1, FAP, αSMA, PDGFRβ and Caveolin1. Similar to Ohlund *et al.'s* iCAF population, one of these subsets also regulates the immune microenvironment, inducing regulatory T cell differentiation. In combination with more recent scRNAseq studies, it is now evident that the pathogenic properties of fibroblasts, previously assumed to be ubiquitous, such as ECM remodelling, antigen-presentation and vascular support, pertain to specific subpopulations.

Furthermore, distinct fibroblast populations can also dictate the efficacy of cancer therapies. For example, in breast and lung cancer, a discrete IL6 producing population promotes resistance to chemotherapy, by enhancing cancer stem-cell survival [33]. Similarly, a

subset of CAFs in breast cancer, reduces expression of the oestrogen receptor on malignant cells, diminishing their sensitivity to tamoxifen [34]. Moreover, further investigation of the immune modulating population identified by Costa *et al,* uncovered additional functional subsets which resemble both the iCAF and myCAF phenotypes. Interestingly, iCAF clusters were associated with CD8+ T cell infiltration and a positive response to immunotherapy, whereas myCAFs were associated with resistance to therapy [35]. This association may be mediated by the ability of myCAFs to promote Treg differentiation and upregulation of immune-checkpoint molecules.

Discrete pathogenic populations are also present in chronic inflammatory disease. Indeed, the functional properties of fibroblast-like synoviocytes (FLS) in rheumatoid arthritis (RA) are determined by their anatomical location in the joint [36]. The synovial membrane can be divided into two distinct mesenchymal structures. This includes an epithelial like membrane, known as the lining layer (LL), which is composed of fibroblasts and macrophages, as well as the underlying sublining (SL), where endothelial cells are located [36,37]. While both compartments are disrupted in disease, the SL alone becomes infiltrated with leucocytes and greatly expands [38]. LL and SL fibroblasts can be identified by distinct marker repertories; while Prg4, Cd55 and Clic5 mark the LL population, Thy1 and CD34 define various subsets within the SL [36,39]. Furthermore, recent finding in both mice and humans, reveal that FLS in the LL display tissue destructive transcriptional signatures and are likely responsible for bone and cartilage damage. In contrast, SL fibroblasts display a pro-inflammatory signature and are characterised by expression of Thy1, PDPN and FAP [36,39]. These functional programs were validated by adoptive transfer of Thy1- and Thy1+ populations in murine models. Here, Thy1+ populations promoted leukocyte accumulation and inflammation, while Thy1- fibroblasts promoted damage and bone erosion [36].

Analogous to RA, pathogenic subsets have also been uncovered in ulcerative colitis (UC). However, owing to the large differences in the number of cells sequenced, their characterisation is hampered by considerable variability. Nevertheless, across these datasets, the spatially distinct populations identified in normal gut tissue, are also present in disease [13,16,17]. Upon exposure to inflammatory stimuli, fibroblasts in the lamina propria adopt an activated THY1+ PDPN+ FAP+ inflammatory phenotype, resembling SL fibroblasts in RA, which expands in UC [13]. Furthermore, owing to a high number of sequenced cells,

Smilie et al. were able to distinguish this THY1+ PDPN+ FAP+ population from a distinct WNT2B+ RSPO3+ CCL19+ CCL21+ population [13]. The latter is thought to interact with LRG5 expressing intestinal stem cells and is associated with poor prognosis in colorectal cancer.

2.3 Common themes across anatomical sites and diseases. Although a diverse array of functional subsets have been described, a re-occurring theme across both disease and tissue is the identification of two main phenotypes: -'immune-interacting'and 'tissue remodelling'. Thus, the iCAF and myCAF populations defined by Ohlund *et al,* may represent a shared paradigm [23]. Here, inflammatory fibroblasts express cytokine and chemokines, indicating an important role in attracting and maintaining immune cells. This may aid persistent inflammation in RA, inflammatory bowel disease (IBD) and cancer. However, a separate subset produces and activates connective tissue components and remodelling enzymes. While these properties are critical for tissue repair, dysregulation in disease leads to pathological matrix remodelling in cancer and fibrosis. The identity of this remodelling population is dependent upon the local tissue ecosystem. For example, fibrosis is not a common feature in RA, thus, remodelling in this context refers to the interactions between LL fibroblasts and the surrounding cartilage and bone. Like fibrosis and cancer, aberrant remodelling driven by this population, causes significant tissue damage. **(Figure 1)**

Interestingly, across disease, these populations are induced and maintained by similar signalling networks. For example, NFκB and the JAK/STAT pathways are key regulators of the immune-interacting phenotype [40–45]. Activation of these pathways is induced by a variety of signals, including sensing of pathology and damage by PAMPs , such as Toll-like receptors, as well as inflammatory cytokines such as TNF and IL1B [4–6,46–50]. Indeed, the $TNF^{\Delta ARE}$ mouse model, in which TNF overexpression leads to RA- and Crohn's-like-pathology, showed that synovial and intestinal fibroblasts are necessary and sufficient targets of TNF, orchestrating both pathologies [51]. Furthermore, in both cancer and RA, initial stimulation by inflammatory factors, is sustained by an autocrine positive feedback loop. Here, activation of the NFκB in fibroblasts, leads to production of LIF. This, in turn, activates the STAT pathway, augmenting their inflammatory signalling network [40,52]. Fibroblasts in IBD also lie at the centre of a positive feedback loop, engaging in paracrine interactions with adjacent leukocytes. Here commensal bacteria induce production of TNFα and Il1B in innate immune cells, such as monocytes, which elicits a pro-inflammatory phenotype in local fibroblasts and leads to

further immune recruitment [53]. Similarly, the JAK/STAT upstream activator oncostatin M, also promotes expression of pro-inflammatory factors in peripheral tissue fibroblasts and is associated with anti-TNF resistance in IBD [54,55] .

While pro-inflammatory cytokines appear to regulate the fibroblast immune-interacting phenotype, TGFβ production and matrix stiffness are integral for the induction of myofibroblast differentiation [56,57]. Specifically, in cancer, the development of a fibrotic matrix also establishes a positive feedback loop to maintain the myCAF phenotype. Here, increased matrix rigidity activates YAP/TAZ signalling, enhancing fibroblast contractility, which further stiffens the surrounding ECM [56]. In addition, in fibrosis, the wnt pathway has also been shown to promote myofibroblast activation and expansion [18]. However, factors driving the acquisition of tissue remodelling and damage in RA remain elusive.

The stromal niche varies from organ to organ, depending on the type of structural cells present, and plays an important role in the activation of both immune-interacting and remodelling fibroblasts. This was recently demonstrated by Wei et al. who elegantly showed that Notch ligands JAG1 and DLL4, expressed on the surface of endothelial cells, engage Notch3 on immune-interacting fibroblasts, inducing their expansion in disease [37]. Conversely, an almost identical interaction between endothelial cells and hepatic stellate cells in liver fibrosis, induced a pathogenic remodelling phenotype [58]. This highlights the importance of disease and tissue context in determining the outcome of pathogenic signalling networks. (**Figure 1**)

Furthermore, such intrinsic differences in tissue and disease states determine the presence and proportion of remodelling and immune-interacting fibroblasts. . Although these subsets exist in tandem across many types of cancer and arthritis, this dichotomy may not be universal. For example, remodelling fibroblasts are the dominant population in liver and lung fibrosis, while immune-interacting subsets appear to monopolise UC. , [13,17,18,22,58]. In addition, inflammatory fibroblasts described by Kinchen et al also express the matrix cross-linking enzyme LOX, which suggests that these two phenotypes may not be mutually exclusive [17]. However, this subset does not express the full complement of genes associated with matrix remodelling populations in fibrosis and cancer. Thus, a true myCAF counterpart,

in UC, likely remains elusive**.** Interestingly, Biffi et al. demonstrate plasticity between the iCAF and myCAF states, which are regulated by the surrounding soluble milieu [40]. In this system, TGFβ disrupts NF-kB signalling, cause fibroblasts to transition from iCAFs to myCAFs. Similarly, activation of the transcription factor PU.1 promotes matrix remodelling properties in fibrosis, even in the presence of TNF [59]. This plasticity may explain the expression of both inflammatory and remodelling genes in a single population. However, it also raises the fundamental question of whether immune-interacting and remodelling populations, across tissue and disease, exist in a similar polarised system or develop from distinct pre-existing populations due to differential cues. ***(Figure 2).***

3.0 Fibroblasts as immune regulators

A successful inflammatory reaction relies on a careful balance of immune recruitment, activation and resolution. Indeed, perturbation of this equilibrium leads to aberrant immunity, which can exacerbate disease pathology. The conservation of immune-interacting fibroblasts, across anatomical location and disease, highlights their pivotal role in local tissue immunity. Their immunological properties range from recruitment and activation of immune cells, to immunosuppression and removal of inflammatory infiltrates. This reflects inter-tissue heterogeneity, which plays an important role in determining the local immune response. Here, expression of particular surface molecules and leukocyte recruitment factors, enable fibroblasts to communicate their anatomical location to circulating immune cells. Known as the 'stromal address code', unique combinations of signalling molecules dictate the identity of recruited leukocyte populations and the behaviour appropriate for the surrounding tissue [60]. For example, naïve cells are recruited to secondary lymphoid organs, whereas memory cells are recruited to peripheral tissues [60]. By governing recruitment, activation and removal of immune cells, fibroblasts act as custodians of immunological balance, tipping the scales from controlled immunity to persistent and un-resolving inflammation, or to an immunosuppressive environment. In the following sections we will explore how the fibroblast command the immune response in infection, inflammation, and cancer.

3.1 ***Immune regulation in lymphoid tissue.*** While the importance of fibroblast-immune interactions in peripheral tissues is rapidly emerging, their role in primary and secondary lymphoid organs (SLOs) has long been appreciated. Lymphoid tissue, such as bone marrow, lymph nodes, Peyer's patches and splenic white pulp, control the appropriate differentiation and release of circulating leukocytes, as well as enable innate and adaptive cells to converge and converse [61]. This is reflected in the stromal address code of local fibroblasts which, through a network of soluble factors and adhesion molecules, coordinate the position, preservation and phenotype of immune cells within these tissues. For example, bone marrow fibroblasts primarily function to constrain immature cells, until differentiation occurs and release into the blood is appropriate. This is mediated by the secretion of CXCL12 and expression of the adhesion molecule VCAM,1 which bind CXCR4 and integrin α4β1 respectively. However, bone marrow fibroblasts also promote B-cell maturation, a process mediated by a separate, Il7 producing population [62,63].

Conversely, SLOS are instrumental for the activation of acquired immunity [64]. Similar to peripheral organs , the functional organization of classical SLOs is underpinned by highly specialized fibroblastic stromal cells, which are usually referred to as fibroblastic reticular cells (FRCs). Recent scRNAseq analyses have categorized and elaborated phenotypical differences of FRC subsets [65–68] . Broadly, the particular anatomical location and the immune cells they interact with, determines the FRC subset identity. For example, T cell zone reticular cells (TRCs), are located in the paracortex where they orchestrate T-cell priming by antigen-bearing dendritic cells (DCs). This involves recruitment, homing and maintenance of naïve T cells, via secretion of chemoattractants CCL19, CCL21 and CXCL12, and the lymphocyte growth factor Il-7 [69–71]. B cell-interacting reticular cells (BRCs, including the follicular dendritic cells (FDCs) of the germinal centre), produce CXCL13, to recruit and maintain a pool of naïve B cells [72,73]. Conversely, marginal reticular cells (MRCs) are situated between the subcapsular sinus and B cell follicles. Here, they regulate local macrophages and lymphatic endothelial cells, as well as acting as a source of FDCs during the formation of germinal centres [74,75]. Other FRC populations include medullary reticular cells (MedRCs) and perivascular reticular cells (PRCs) [64].

Importantly, FRCs also employ several mechanisms to promote tolerance to self-antigens and regulate immune responses against commensal bacteria. This involves activating

regulatory T cells, as well as coordinating a suppressive soluble environment, through the enzymatic activity of indoleamine 2,3 dioxygenase (IDO) and cyclooxygenase-2 (COX2) [76]. Moreover, FRCs can directly present antigen to T and B cells, which both activates adaptive immune responses, as well as deletes or induces dysfunction of self-reactive lymphocytes [77,78]. Thus, the stromal address codes of fibroblasts in lymphoid tissue is centred around regulation of innate lymphoid cells, myeloid cells, as well as recruitment, activation and retention of lymphocytes. However, it also includes mechanisms of immune suppression to maintain homeostasis.

3.2 ***Coordinated immune responses to infectious agents.*** Immune surveillance is enforced by the recognition of pathogens and the well-orchestrated activation of innate and adaptive immune responses in SLOs. Like peripheral tissues, FRC subset identity in homeostasis, is preserved in the inflamed state [66,68]. However, viral infection reprograms FRC properties to direct innate and adaptive immune cell migration and differentiation. This includes FRC-generated chemokine gradients to localize proliferating B cells to the T-B border, thereby causing stretching of the BRC network to create the germinal centre dark zone [79]. Moreover, FRCs in lymph nodes draining the site of viral infection profoundly change the expression patterns of genes involved in antigen presentation, extracellular matrix, chemokine and cytokine signalling [80]. Again, reflecting their peripheral counterparts, FRC properties are regulated by distinct cues from interacting immune cell partners. For example, T helper cells-derived IL-17 locally activates TRC and may metabolically support fibroblast proliferation and survival [81].

Crucially, after pathogenic clearance, FRCs aid the return to lymph node homeostasis. This is mediated by promoting lymphangiogenesis, via transporting VEGF to lymphatic vessels, which facilitates removal of lymphocytes [82]. In addition, during an immune response, LNs swell to accommodate T cell expansion. This is enabled by FRCs, which reduce contractile tension in the reticular network. Upon resolution, FRCs contract this network and become quiescent, allowing lymph node shrinkage [76]. Indeed, acute transcriptional reprogramming of lymph node FRCs is transient [66], as gene expression profiles following removal of the viral infection are similar to those recorded in naive mice [68]. Thus, the degree of inflammation-induced transcriptional remodelling reflects the dynamic nature of FRC-immune cell

interaction, that varies temporally, and is indicative of the adaption of the particular immune environment to different pathogens.

Besides the role of FRCs in the functional organization of SLOs during infection, tissue-resident fibroblasts also exert physiological functions in direct response to microbial signals. This includes activation of Cox2/PGE2 [83], NF-kB, MAPKs and the inflammasome [84]. Moreover, fibroblasts directly recognise microbes via TLRs and activation of MyD88, instigating tumour regulatory responses [47]. In intestinal chronic inflammation and fibrosis, specific microbiota may be required for TL1A-mediated fibroblast activation and transformation to myofibroblasts [85]. Additional evidence is now beginning to emerge that microbial metabolites are also directly sensed by fibroblasts and modulate inflammation and fibrosis in mice [86].

3.3 ***Skewing immunological balance towards persistent inflammation.*** Chronic inflammation is characterised by persistent leukocyte retention, in the absence of resolution and repair. During resolution, immune populations are removed from peripheral tissues by reducing survival cues, upregulating apoptotic signals and increasing lymphatic drainage. Disruption of these processes, as well irregular chemokine gradients, results in the capture and accumulation of inflammatory cells. Local fibroblasts drive this disorder by altering their stromal address code to reflect FRCs in lymphoid tissue, leading to recruitment and retention of leukocytes.

This begins with the recruitment of myeloid cells, highlighted in a recent study by Martin et al. Here, they discerned a population of activated fibroblasts in Crohn's disease, expressing CCR2 ligands; CCL2 and CCL7 [87]. Ligand-receptor analysis confirmed an interaction between these fibroblasts and a population of monocytes expressing S100A8, S100A9 and S100A4. Furthermore, scRNAseq from paired PBMCs indicated this population migrates from peripheral blood into tissue. Concurrent expression of ACKR1 on activated endothelial cells was suggestive of interplay between fibroblasts and endothelium, which enables transcytosis of inflammatory monocytes.

Although myeloid infiltration in a common feature of inflammatory disorders, it is the switch from innate to adaptive immunity that demarcates the development of persistent

inflammation, correlating with worse outcomes and poor treatment response [88,89]. Fibroblasts aid this transition by recruiting and maintaining lymphocyte populations in peripheral tissues. For example, in UC, the WNT2B+ RSPO3+ C3+ subset expresses lymphocyte recruitment factor, CCL19 [13]. Similarly, FLS in RA upregulate CXCR4 on the surface of T cells, to attract and retain lymphocytes within the tissue [90]. Once locally confined, FLS promote lymphocyte survival, proliferation and activation by production of type 1 interferons, IL-6, B cell activating factor (BAFF) and A Proliferation Inducing Ligand (APRIL), as well as direct antigen presentation [5,91–94].

Crucially, whilst homeostasis in lymphoid tissues is restored after pathogenic infection, fibroblasts in persistent inflammation sustain lymphocyte retention. This may reflect the inability of inflammatory fibroblasts to return to a homeostatic state. Indeed, these cells are resistant to apoptosis, and retain pathological features in the absence of disease. For example, when implanted into healthy tissue of severe combined immunodeficient mice, cultured RA fibroblasts, but not osteoarthritis fibroblasts, recapitulate aspects of disease such as local cartilage invasion [95]. In addition, FLS isolated from RA, showed an increased propensity for activation of inflammatory pathways, upon re-stimulation (reviewed [96]). Akin to 'positional identity', this may be mediated by epigenetic modifications, such as DNA methylation. Indeed, methylation patterns are altered at early stages of inflammation, and continue to change as the disease advances [97–99]. The stability of these changes *in vitro,* suggests that inflammatory disease imprints a pathological phenotype onto local fibroblasts, preventing reversion to a homeostatic state [100] . ***(Figure 3)***

3.4 ***Organisers of tertiary lymphoid structures*** Chronic inflammation necessitates the presence of an immune outpost in peripheral tissue to recruit and sustain lymphocytes. Thus, in addition to attracting and restraining leukocyte populations, fibroblasts also acquire functional and phenotypic properties to facilitate the formation of tertiary lymphoid structures (TLS), which perpetuate the immune response [101]. These ectopic sites of lymphoid neogenesis, consist of an organised structure of lymphocytes (B cell and naïve CCR7+ L-selectin+ T cells), myeloid cells (DAMP3+ DCs) and stromal cells.

Ectopic lymphoid neogenesis resembles development of SLOs, which are dependent on the interplay between mesenchymal lymphoid tissue organiser (LTo) cells and haemopoietic lymphoid tissue inducers (LTi) cells, via the LTβR signalling pathway [102]. In the context of persistent inflammation, it is likely that priming of a stromal cell occurs through chronic exposure to pro-inflammatory cytokines such as members of the TNF family, IL-4-7, IL-13, IL-15, IL-17, IL-22, IL-23 and IL-27 with varying degrees of dependence on RORγ [70,103–108]. The source of these factors, and thereby the counterpart of the LTi in ectopic lymphoid neogenesis, are innate immune cells, such as myeloid cells and granulocytes [101,109,110]. Following priming, subsequent organisation of TLS by the LTo is partially dependent on LTBR and TNF signalling and activation of the NF-kB cascade [67,111–113]. LTαβ/LTβR signalling and expression of chemokines, establishes organised zones of B and T cells. Vascular regions in the T cell zones differentiate into structures resembling high endothelial venules (HEV). Upregulation of peripheral node addressins in these regions allows recruitment of CCR7+ L-selectin+ naïve T cells. Across anatomical locations, TLS preferentially form near vascular structures and the corresponding LTo cells include a variety of stromal cells, such as αSMA+ fibroblastic cells, myofibroblasts and pericytes [114,115]. Thus, both the priming of the LTo stromal cell and the corresponding spatial distribution of the TLS is tailored to the surrounding proinflammatory milieu and tissue type.

The effector function and clinical implications of TLS in disease are the subject of much debate. Although some studies postulate that CXCL13 and ectopic lymphoid neogenesis, are drivers of inflammation in rheumatoid arthritis, correlating with poor clinical outcomes, this is disputed by others [116–121]. In IBD, CCL19+ CCL21+ stromal cells have recently been discerned in single cell analysis [13,17,87], potentially representing LTo-like cells. However, it is still too early to conclude whether TLS are simply associated with IBD, as opposed to a key driver of inflammation. On the other hand, in Sjogren's syndrome (SS), there is a clinical association with poor prognosis. Here, TLS promote autoantibody production, potentially driving expansion of malignant autoreactive B cell clones [108,122,123].

Conversely, in cancer, TLS are associated with improved prognosis (reviewed by Fridman [124]) and have recently been demonstrated to heighten the efficacy of immune checkpoint inhibitors [125]. This dichotomy reflects key differences between the immune microenvironment of chronic inflammatory disorders and malignancy. While the former is

characterised by recruitment and retention of pro-inflammatory immune populations, tumours cultivate an immunosuppressive niche to enable their growth [126]. Although immune cells can detect neo-antigens produced by genetically unstable tumours [127,128], the malignant microenvironment dampens their activity. This is mediated by recruiting regulatory cells, inducing suppressive phenotypes or actively excluding leukocytes (reviewed [129]). Restoration of effector functions by immunotherapies has led to immune-mediated destruction of tumours, in melanoma, lung, renal and head and neck cancer [130–133], illustrating the importance of a robust immune response in determining malignant progression. Similar to chronic inflammatory disorders, TLS in cancer are composed of dendritic cells, T and B lymphocytes, as well as HEVs [134–136]. It is thought that TLS promote anti-tumour immunity by facilitating DC antigen presentation, enhancing cytotoxicity and promoting T-cell mediated B cell differentiation [137–139]. In turn, plasma B-cells produce antibodies against tumour antigens and are thought to directly present antigen to CD8 T-cells, further augmenting their activity [139–142]. While robust T-cell responses are associated with TLS formation [136,137], it remains unclear whether TLS truly heighten anti-tumour immunity or are merely an indication of a functional immune response ***(Figure 4)***.

3.5 ***Promoting immunological tolerance in cancer.*** While little is known about the role of stromal cells in TLS formation in cancer, CAFs are intimately involved in immune recruitment and regulation in the malignant microenvironment. Thus, their stromal address code may determine the potency of the anti-tumour immune response, dictating disease outcome. Similar to FLS and gut fibroblasts, CAFs also acquire properties associated with the lymphoid mesenchyme. A key component of the iCAF inflammatory secretome, in PDAC and melanoma, are factors that act to recruit and regulate myeloid cells [28,29]. Indeed, across malignancies, CAFs attract and maintain tumour associated macrophages (TAMs) via secretion of CCL2, CCL5, CXCL1, CXCL12, IL-6 and Chitinase-3 like 1 (Chi3L1) [45,143–148].

However, again, it is the presence of lymphocytes that determines prognosis [148–150]. In contrast to fibroblasts in inflammatory disease, CAFs have been shown to exclude lymphocytes and mimic the suppressive properties of FRCs, promoting tumour immune evasion. While CAFs also adopt the CXCL12-CXCR4 axis to restrain T cells, instead of amassing lymphocytes in the tissue, the peripheral location of CAFs in PDAC, prevents T cell

infiltration [151]. Tumour fibroblasts also suppress T cell activity via production of soluble cues, such as PGE2, and immune checkpoint ligands PDL1 and PDL2 [152,153]. In addition, while CAFs are able to present antigens, this interaction is utilised to suppress and delete CD8 T-cells. Akin to the mechanisms of self-tolerance in the LN, FasL and PDL2 on the surface of CAFs, repress T-cells in an antigen dependent interaction [153]. CAFs further promote a tolerogenic environment by recruiting and restraining regulatory T cells, via CXCL12 and adhesion molecules OX40L and JAM2, as well polarising myeloid cells [27,145]. This includes inducing immunosuppressive phenotypes in TAMs, by secreting CXCL12 and Chi3L (Cohen et al., 2017; Comito et al., 2014) [144,145], and reducing the ability of DCs to present antigens and activate T cell responses. This is mediated by production of the metabolite tryptophan metabolite kynurenine bytyptophan-2,3 dioxygenase, which downregulates costimulatory molecules on DCs, as well as promoting expression of regulatory cytokines TGFβ and IL10 [154]. Similar to inflammatory diseases, maintenance of this unique immune microenvironment, may be driven by epigenetic imprinting of tumour fibroblasts. Indeed, CAFs display discrete DNA methylation patterns, which maintain their pathological properties [155–157]. . Thus, it is possible that while fibroblast epigenetic remodelling enables persistent inflammation in chronic inflammatory disorders, in cancer, it may promote continuous tumour immune privilege.

4. *Importance of location and disease in fibroblast function*

The relationship between tissue specificity and pathological cues in sculpting fibroblast phenotype and heterogeneity in disease, is a source of much debate. On one hand, tissue-specific transcriptional programs shape the identity of local functional subsets. This influences the composition of populations in pathology and their associated functions, which may explain the predisposition of anatomical sites to certain types of diseases. For example, viral tropism (skin, gut, brain), chronic inflammatory diseases (gut, joints, skin) and metastatic spread to certain tissues (bone marrow, lung, lymph node). Tissue imprinted functional properties are particularly pertinent in the tumour microenvironment, owing to the multiple origins from which CAFs may be derived. As well as transformation of tissue resident fibroblasts by malignant cues, CAFs also originate from pericytes, tumour cells undergoing epithelial-mesenchymal transition, endothelial cells undergoing endothelial-mesenchymal transition and bone marrow stromal cells that migrate into peripheral tissues

from circulation [158–163]. Interestingly, a recent study in breast cancer highlighted different functional properties associated with recruited bone marrow fibroblasts, compared to tissue residents CAFs [164]. This indicates that fibroblasts from different tissues retain functional programmes even when exposed to a new local environments.

On the other hand, the relative composition of fibroblasts can change depending on the pathological insult. For example, in the synovium, inflammatory populations are enriched in leukocyte-rich RA, while populations involved in tissue damage are more prevalent in osteoarthritis [39]. Similarly, in breast cancer, a distinct immune-regulatory CAF population is enriched in triple negative, compared to luminal tumours [27]. This indicates that while certain features are imprinted, some plasticity is retained. Given that they are embedded in a network of immune, epithelial and endothelial populations, fibroblasts can adapt their properties and address codes to complex paracrine cues from surrounding cells.

This highlights the importance of bioinformatic tools such as CellPhoneDB and NicheNet to interrogate single cell sequencing data [165,166]. These systems produce a ranked list of putative interactions between cell types, creating a powerful system to investigate cell-cell communication, in the heterogeneous environment of disease. Understanding these interactions may enable therapeutic modulation of fibroblast immune-regulatory properties, to promote immune tolerance in chronic inflammation or cytotoxicity in cancer (Table2). However, it is important to bear in mind that bioinformatic insight should be supported by functional evidence and assigning function to groups of cells based on the transcriptome might not be biologically accurate.

5.0 Therapeutic avenues for targeting fibroblasts in disease

An in-depth understanding of the markers that define fibroblast heterogeneity as well as the surrounding tissue ecosystem, is required if fibroblast biology is to transition from bench to bedside (Figure 5). The difficulties associated with selective fibroblast depletion, through targeting FAP in oncology, illustrates the challenges involved. As FAP is highly expressed on CAFs in a variety of cancers, concerted efforts have been made to deplete FAP+ CAFs using immunotoxin, antibodies, DNA vaccines and chimeric antigen receptor (CAR) T cells [167–171]. These strategies were successful in attenuating tumour growth, yet owing to expression of FAP in normal tissues, also resulted in cachexia and anaemia[167,169,171,172]. Furthermore, the

dangers of using single marker approaches to target CAFs without prior knowledge of their functional properties, are illustrated by their depletion in PDAC. Here, depletion of FAP+ CAFs promoted T cell infiltration and synergises with immune checkpoint therapies [151]. However, elimination of αSMA+ CAFs promoted undifferentiated tumour growth and reduced survival [173].

An alternative strategy is to target downstream functions of specific fibroblast subsets. For example, clinical trials are ongoing for the TGFβ inhibitor galunisertib, which aims to reduce CAF matrix production and increase anti-tumour immunity [174,175]. In a similar vein, it is hoped that the CXCR4 inhibitor, AMD3100, will prevent CXCL12 mediated T-cell exclusion and enable lymphocyte infiltration into the tumour [176,177]. Another approach involves targeting the inductive programmes that initiate pathological phenotypes in fibroblasts. In cancer, inhibitors of the FGFR and the vitamin D receptor, aim to broadly reduce CAF activation [178,179]. Interestingly, this was also achieved by administration of tyrosine kinase inhibitors, which were initially designed to inhibit tumour cell signalling. It is thought these modulate fibroblast activity via interactions with PDGFRs and FGFR and has led to the repurposing of nintedanib for treatment of pulmonary fibrosis[180–182].

However, as fibroblasts are composed of functionally distinct populations, it is critical to understand the programs that regulate each population, to enable specific targeting. Furthermore, this strategy raises the possibility of harnessing inductive programs in one disease, to treat another. For example, inhibiting NOTCH3 signalling between endothelial cells and FLS, reduced expansion and reversed the pro-inflammatory phenotype of THY-1+ fibroblasts in RA (Wei et al., 2020). Thus, targeting the NOTCH3 receptor may represent a therapeutic avenue for other chronic inflammatory diseases. Indeed, NOTCH3 is expressed on the THY1+ fibroblasts in the gut lamina propria. Other examples include targeting the NF-kB amplification loop and interactions with inflammatory macrophages.

Additionally, inductive programs that promote immunosuppressive phenotypes in tumour fibroblasts, may also be used to resolve chronic inflammatory disease. However, while we are starting to understand drivers of the iCAF phenotype, upstream factors inducing their suppressive properties are yet to be elucidated. On the other hand, scRNAseq has begun to

shed light on the mechanisms promoting remission of chronic inflammatory disorders. Conditions such as RA and IBD, can be cyclic in nature, in which patients experience periods of remission. In RA, Alivernini et al elegantly demonstrate that fibroblasts in remission adopt tolerogenic and resolving phenotypes, as evidenced by expression of IGFBP5/6 and AXL [183]. This is induced by a unique MerTK+ macrophage population, that is enriched during the remission phase. However, this interaction is bidirectional, as production of GAS6 by THY1^{pos}CXCL14 fibroblasts, activates MerTK signalling in myeloid cells. siRNA abrogation of GAS6, in FLS-macrophage co-cultures, reduces the resolving phenotype of this macrophage subset, reciprocally causing THY1+CXCL14+ fibroblasts to acquire a pro-inflammatory phenotype. This highlights how changes in the local tissue composition, during different disease states, impacts fibroblast properties. Activating these signalling networks in persistent disease may polarise fibroblasts towards a tolerogenic phenotype, ameliorating inflammation.

However, it is important to understand tissue context before modifying cellular signalling or repurposing drugs. This is highlighted by unsuccessful attempts to adopt cytokine blockade strategies from inflammatory rheumatological conditions into treatment of CD. IL-6 trans-signalling was postulated as a key mechanism in mediating resistance of T cells to apoptosis in CD [184]. However, clinical trials investigating IL-6 blockade noted gastrointestinal abscess and perforations in treated patients [185]. This is a known side-effect in patients undergoing anti-IL-6 therapy for rheumatological indications [186]. Similarly, animal models and genome wide association studies implicated a role for IL-17 in the pathogenesis of CD [187–189]. However, not only were inhibitors of the IL-17 pathway ineffective, but they also resulted in worsening colitis [190,191]. In both IL-6 and IL-17 blockade strategies, the pleiotropic nature of the cytokines involved and their impact on the intestinal epithelium was not fully understood. These two cytokines are integral for intestinal tight junctions, through regulation of claudin-2 and occludin respectively [192,193]. As such, whilst negating these pathways were efficacious in rheumatological conditions, blocking them in context of active mucosal inflammation led to adverse events.

6.0 Conclusions and future developments

Fibroblasts are complex multifaceted tissue-resident sentinel cells, shaped by the needs of the surrounding tissue, via epigenetic imprinting during embryogenesis. Upon challenge with a range of different tissue insults, they help to initiate, govern and moderate subsequent immune responses. This includes interaction with granulocytes, myeloid cells, as well as modulating lymphocyte recruitment and retention. If necessitated, particularly in the case of infection, this leads to the creation of immune outposts, in the form of tertiary lymphoid structures. However, in diseases such as chronic inflammatory disorders and cancer, inappropriate fibroblast activation facilitates disease persistence, by induction of pro-inflammatory and immunosuppressive properties respectively. Knowledge of these pathological properties may be harnessed to restore homeostasis across disease. For example, exploiting tolerogenic features may aid resolution of autoimmune disorders, while stimulating an inflammatory response may promote anti-tumour immunity. Thus, unravelling the composition and function of fibroblasts in homeostasis and disease will unlock their therapeutic potential in tissue repair, whilst minimising the risk of adverse effects associated with directly targeting immune cells.

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Figures and tables

Figure 1: Fibroblast heterogeneity in health and disease across tissue

Fibroblast heterogeneity in homeostasis and disease in the gut (A), synovium (B), and lung (C). (A) Fibroblasts are shaped by their position in the crypt axis, with subsets expressing SOX6 and POSTN positioned in close proximity to the epithelium to facilitate epithelial regeneration. In the context of inflammation, the emergence of a THY1+PDPN+FAP+ subset is seen close to the site of mucosal barrier breakdown, orchestrating recruitment of

leucocytes via release of cytokines and appropriate chemokines. (B) The synovium in health and RA. Lining layer fibroblasts produce lubricin to lubricate healthy joints. However, in RA, this population acquires a remodelling phenotype, producing MMPs to break down cartilage and activating osteoclasts to erode bone. iii. In this disease state, the sublining expands and a THY1+ PDPN+ FAP+ population emerges surrounding the blood vessels. Similar to the gut, this population produces inflammatory cytokines. (C) Fibroblast heterogeneity in the healthy and fibrotic lung. AXIN2+ PDGFRA+ fibroblasts reside close to the alveolar niche, where they support stem cell maintenance. A distinct AXIN2+ PDGFRa- population resides closer to the airways. This population acquires a pathogenic remodelling phenotype in disease, promoting fibrosis.

Figure 2: Common mechanisms regulating proinflammatory and remodelling populations across disease.

Two pathogenic fibroblast populations are commonly found across disease: inflammatory and tissue remodelling. These populations are regulated by similar signalling pathways. (A). Inflammatory factors derived from immune infiltrates activate an inflammatory profile in fibroblasts via NFkB. (B) NFkB induces production of LIF, which activates JAK/STAT signalling, propagating NFkB signalling, inducing a positive feedback loop. (C) Inflammatory factors produced by fibroblasts recruit more immune cells, further exacerbating this process. (D) Stromal signals induce expansion of both inflammatory and remodelling populations, including JAG1 and DLL4 activation of Notch3 Notch ligands on endothelium. (E) Physical cues, including matrix stiffening, combine with soluble stimuli to promote the remodelling phenotype. In cancer these phenotypes are interchangeable, depending on the cues that are exposed to.

Figure 3: Transformation of fibroblast functions in inflammation

(A) Environmental insults activate pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) on mononuclear phagocytes and granulocytes, in keeping with an acute inflammatory response. (B) Subsequent release of cytokines such as TNF-alpha or oncostatin M (OSM) from these cells induce a pro-inflammatory phenotype in fibroblasts, that is shaped by local tissue ecosystems. (C) In the setting of chronic inflammation, the local cytokine milieu primes LTo-like stromal cells. This induces expression of chemokines such as CCL19, CCL21 as well CXCL12 and CXCL13. (D) Over time, this LTo-like stromal cell recruits naïve CD4+ cells, DAMP3+ DCs, and follicular B cells to its location, eventually giving rise to a tertiary lymphoid structure.

Figure 4: Fibroblast immune modulation in cancer

Cancer associated fibroblasts (CAFs) have both pro-inflammatory and immunosuppressive properties in the tumour microenvironment. (A) CAFs recruit myeloid cells to the TME by producing cytokines such as CCL2, CCL5, CXCL8, CXCL12, Chi3L1 and IL6. The presence of T-

cells is associated with immune mediated tumour destruction. As such tumours cultivate an an immunosuppressive environment in which, T-cells are actively excluded or held in a dysfunctional state, characterised by expression of exhaustion markers PD1, Lag3 and Tim3 (B). CAFs contribute to T-cell dysfunction by antigen-dependent deletion (C), in which CAFs present antigen via MHCI, while engaging Tcell receptors PD1 and FAS, with PDL2 and FasL. CAFs also actively exclude T-cells from the tumour by producing TGFβ and CXCL12 (D). Finally, CAFs contribute to the tumour immunosuppressive environment by polarising macrophages towards a suppressive phenotype (mediated by CXCL12 and Chi3L1), inducing Myeloid derived suppressor cells (MDSCs, via CXCL12, DKK1 and Il6), as well as reducing ability of dendritic cells to present antigen and activate adaptive immunity (via TDO2/kynurenine, TLSP, IL6).

Figure 5: Harnessing fibroblasts for therapeutics

To therapeutically target fibroblasts in disease, 3 main approaches can be taken. Current therapies have mostly focussed on targeting and deleting FAP+ fibroblasts, using CAR T-cells, DNA vaccines and anti-FAP antibodies (A). An alternate approach is to directly target fibroblast effector functions, such as blocking the action of inflammatory or immunosuppressive factors (B). Finally, to switch fibroblasts from a pro-inflammatory to an immunosuppressive state, or vice versa, inductive programs can be induced or disrupted. Possible target programs are highlighted in (C).

Tables

Table 1. Common mechanisms of fibroblast activation across tissue and disease

Table displays mechanisms that activate pathogenic properties in fibroblast in disease. Disease type and tissue of origin are indicated.

Mechanism of Activation	**Disease**	**Organ**	**References**
TLRs			
TLR2	Rheumatoid Arthritis	Joints	[4]
TLR3	Rheumatoid Arthritis	Joints	[5,6,46]

TLR4	Liver fibrosis Rheumatoid Arthritis Intestinal Cancer	Liver Joints Gut	[49] [48] [47]
TLR5	Intestinal Fibrosis (UC/Crohn's)	Gut	[50]
Cytokines			
TNF	Rheumatoid Arthritis Inflammatory Bowel Disease Cancer	Joints Gut Colorectal liver metastasis	[46,51] [51] [194]
OSM	Inflammatory Bowel Disease	Gut	[55]
IL17	Fibrosis	Liver Skin Gut	[195] [196] [197]
IL1B	Cancer	Skin Pancreas	[42,198] [42]
Il1a	Cancer Rheumatoid arthritis	Pancreas Skin Joints	[40] [198] [46]
Mechanical Force	- Wound healing Cancer	Periodontium Skin Liver Heart Breast	[199] [200] [201] [202,203] [56,204]
Signaling Pathways			

NFkB	Cancer Rheumatoid Arthritis	Gut Skin Pancreas Joints	[43] [20,42] [40,42] [41,44,205]
STAT3	Cancer	Pancreas Liver	[40] [45]
STAT4	Rheumatoid Arthritis	Joints	[52]
STAT1	Rheumatoid Arthritis	Joints	[44]

Table 2. Common mechanisms of immune regulation

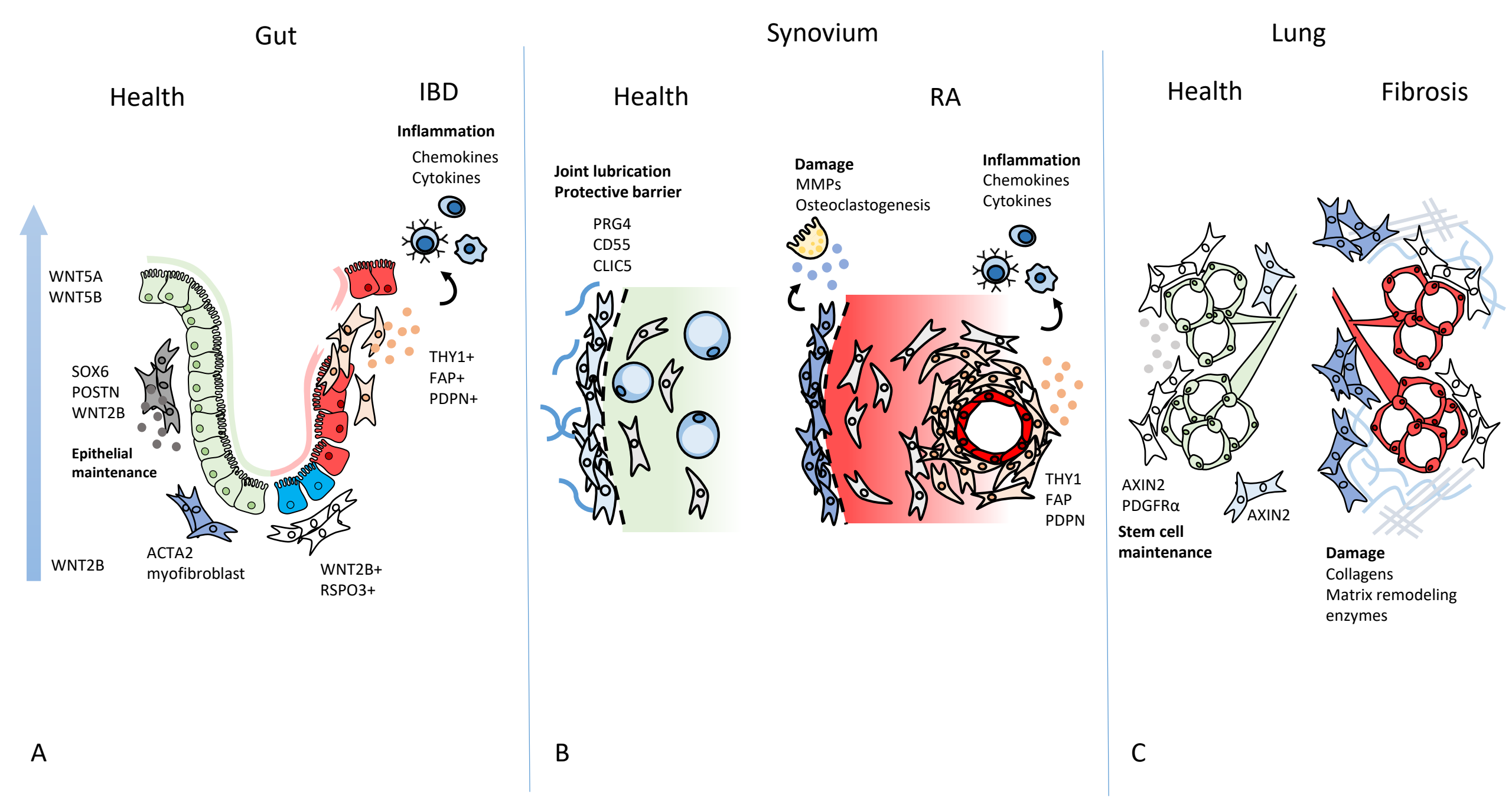
Table displays common mechanisms through which fibroblasts regulate immune recruitment, activation and suppression, in disease. Disease and anatomical site are indicated.

Mechanism of Immune Regulation	**Disease**	**Organ**	**References**
Antigen presentation			
MHCI	Cancer	Skin	[153]
MHCII	Inflammation: RA Cancer	Joints Pancreas	[91,206] [29]
Attraction of Myeloid Cells			
CCL2	Inflammation: RA Inflammation: IBD Cancer	Joints Gut Breast Liver	[207] [87] [147] [45]

		Colon	[143]
CCL5	Inflammation: RA	Joints	[207]
CCL8	Inflammation: RA	Joints	[207]
CXCL1	Inflammation: IBD Cancer	Gut Colon Lung Breast Skin Pancreas	[87] [208] [208] [208] [208] [146]
CXCL2	Inflammation: IBD	Gut	[13]
CXCL5	Inflammation: RA	Joints	[207]
CXCL8	Inflammation IBD	Gut	[13]
CXCL10	Inflammation: RA	Joints	[207]
CXCL12	Cancer	Prostate	[145]
Chi3L1	Cancer	Breast	[144]
Il6	Cancer	pancreatic	[23]
Immunosuppression			
CXCL12 (Macrophages, MDSC)	Cancer	Prostate Liver	[145] [209]
DKK1 (MDSC)	Cancer	Skin Lung	[210] [210]
Il6 (MDSCs, DCs)	Cancer	Pancreas Liver	[211] [212]
TDO2/Kynurenine (DCs)	Cancer	Lung	[154]
TLSP (DCs)	Cancer	Pancreas	[213]

FASL (T-cells)	Cancer	Skin	[153]
PDL1 (T-cells)	Cancer	Skin Pancreas	[198] [152]
PDL2 (T-cells)	Cancer	Skin Pancreas	[153,198] [152]
PGE2 (T-cells)	Cancer	Skin Pancreas	(Khalili et al., 2012)(Khalili et al., 2012) (Gorchs et al., 2019)(Gorchs et al., 2019)
Chi3L1 (Macrophages)	Cancer	Breast	[144]
SASP:CCL8, CXCL5, CCL2, CCL7, Il6, CXCL1, CXCl14, CCL5 (MDSC)	Cancer	Skin	[214]
T-cells Attraction/retention/ Exclusion			
CXCL12	Inflammation: IBD Cancer (Exclusion)	Gut Pancreas	[215] [151]
TGFB	Cancer (Exclusion) Inflammation: RA (Attraction)	Bladder Colon Joints	[175] [216] [90]
OX40L	Cancer (retention of Tregs)	Breast Ovary	[27] [217]
JAM2	Cancer (retention of Tregs)	Breast HGSOC	[27] [217]
CCL19	Inflammation: IBD	Gut	[17]
CCL21	Inflammation: IBD	Gut	[17]

Endothelial Adhesion			
Upregulation VCAM	Inflammation: RA	Joints	[218]
Upregulation ICAM	Inflammation: RA	Joints	[218]
Survival Factors			
CXCL12	Inflammation: RA	Joints	[219]
VCAM	Inflammation: RA	Joints	[219,220]
BAFF	Inflammation: RA	Joints	[5]
APRIL	Inflammation: RA	Joints	[5]
Type1 interferons	Inflammation: RA	Joints	[221]
Other			
Il6 (Th17 differentiation)	Cancer Inflammation: RA	Lung Joints	[222] [223]

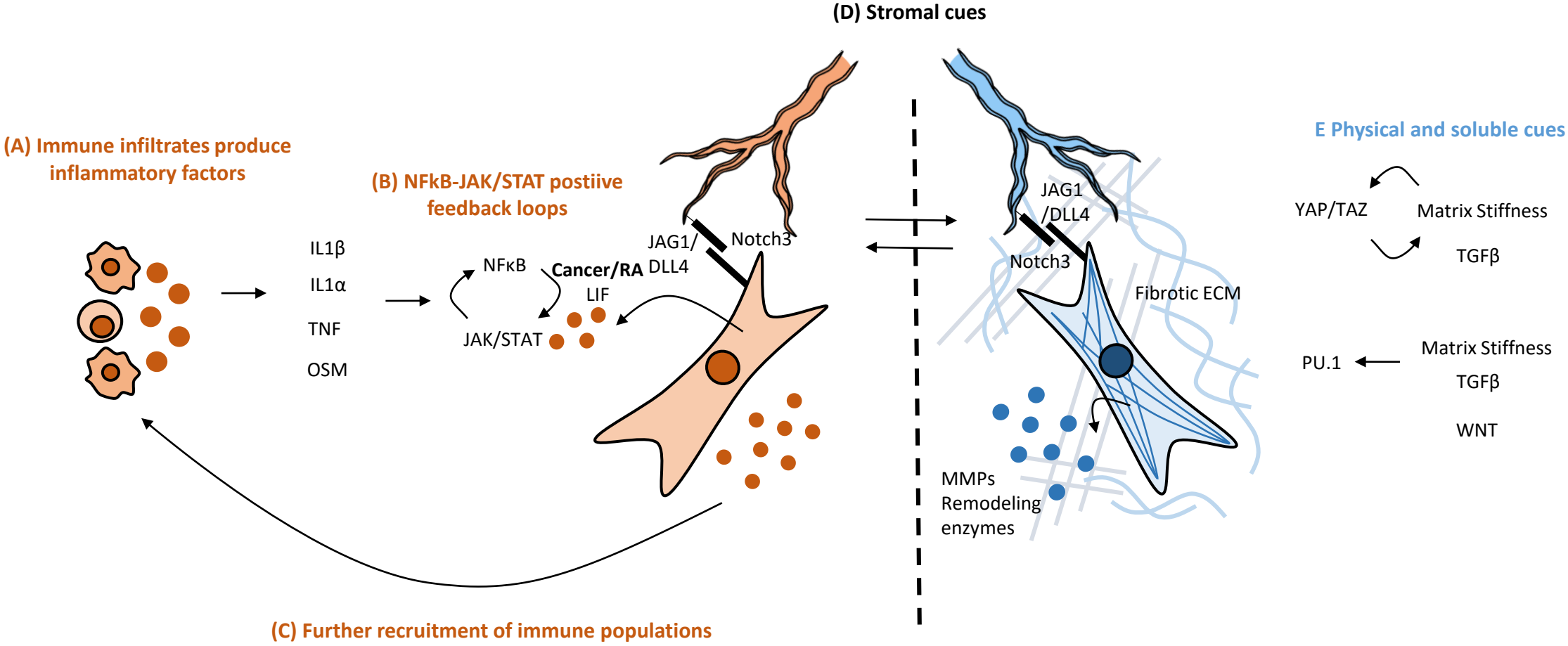

Gut
Health
IBD
Inflammation
Chemokines
Cytokines
WNT5A
WNT5B
SOX6
POSTN
WNT2B
Epithelial maintenance
THY1+
FAP+
PDPN+
WNT2B
ACTA2
myofibroblast
WNT2B+
RSPO3+
A
Synovium
Health
RA
Joint lubrication
Protective barrier
PRG4
CD55
CLIC5
Damage
MMPs
Osteoclastogenesis
Inflammation
Chemokines
Cytokines
THY1
FAP
PDPN
B
Lung
Health
Fibrosis
AXIN2
PDGFRα
Stem cell maintenance
AXIN2
Damage
Collagens
Matrix remodeling enzymes
C

Immune-interacting fibroblasts:

Cancer, RA and IBD: Cytokines and chemokines

Tissue Remodeling fibroblasts:

Fibrosis & Cancer: Fibrotic ECM proteins, MMPs, cross-linking enzymes
RA: Cartilage destruction and promotion of osteoclastogenesis



Acute inflammation

Chronic inflammation

Environmental stressors

A

- Mononuclear phagocytes
- Granulocytes

TNF-alpha
OSM
IL1a

B

IBD

CXCL1
CXCL2
CXCL5
CXCL6
IL11
IL24
FAP

RA

IL6
LIF
IL33
IL34

THY1+ PDPN+ FAP+ (activated fibroblast)

C

IL4 IL15
IL5 IL17
IL6 IL22
IL7 IL23
IL13 IL27

+/- RORγ

CXCL12
CXCL13

CCL19
CCL21

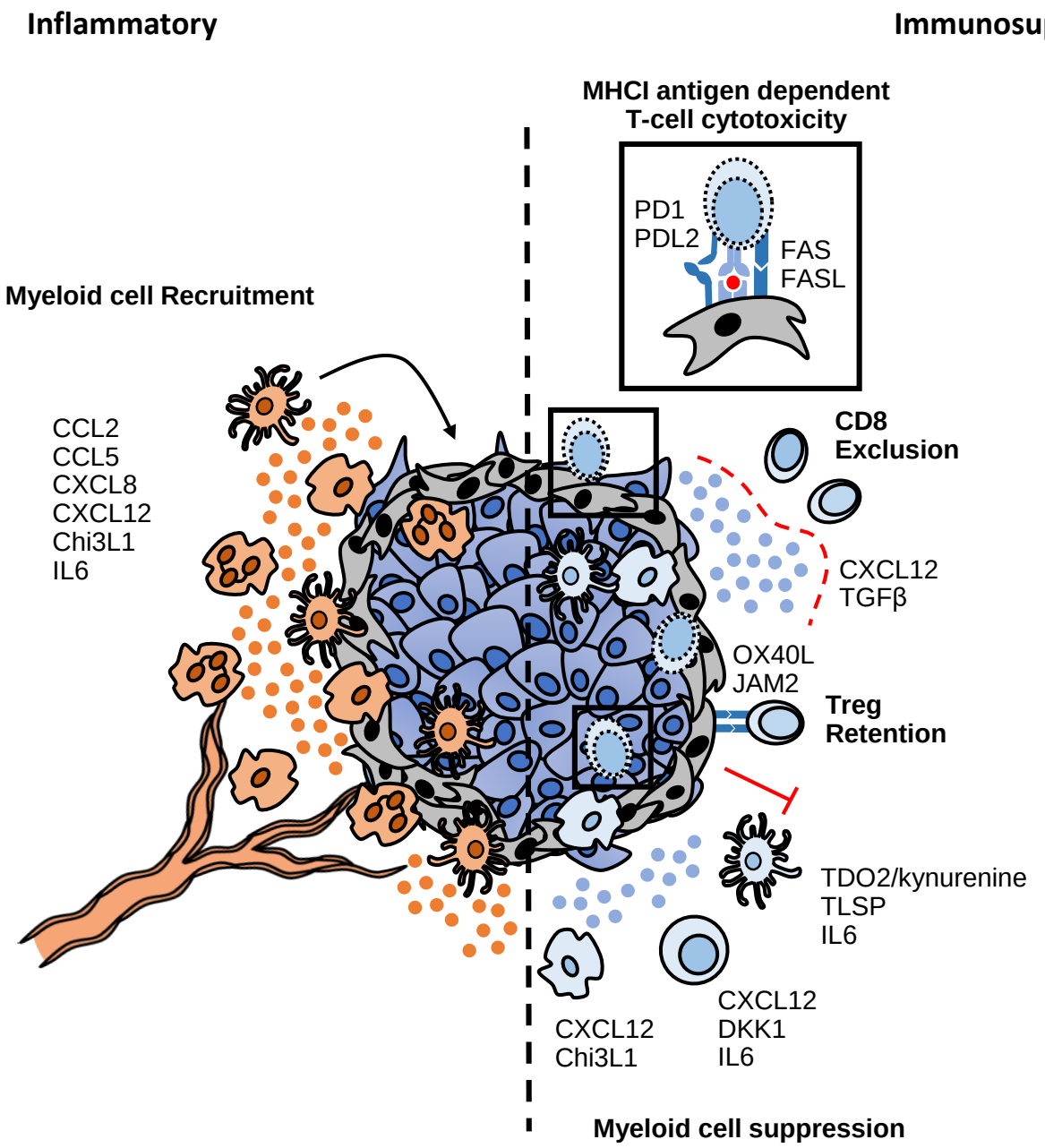
LTo-like stromal cell

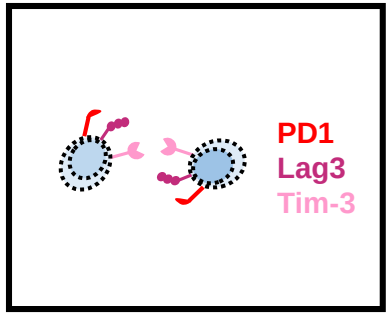
D

LTαβ/LTβR signalling

Tertiary lymphoid structure

Recruitment of naïve CD4+, DAMP3+ DCs, and follicular B cells and formation of PNaD structures

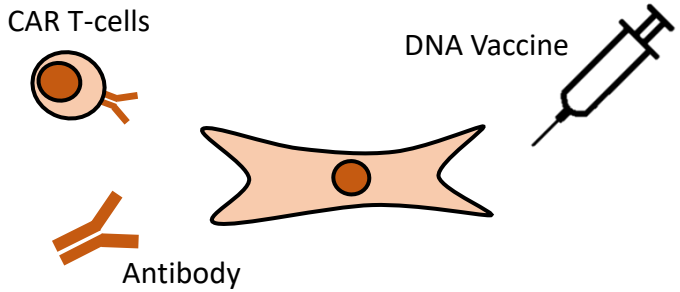




T-cells in the TME are dysfunctional and express exhaustion markers

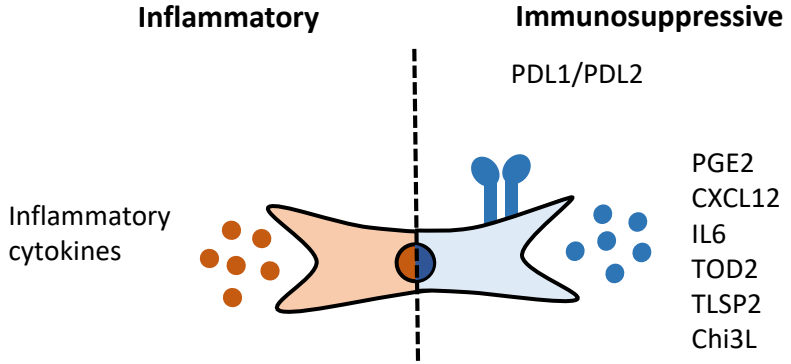
A.

Cell based approach



B.

Effector approach



C.

Inductive program approach