Stromal-Immune Cell Interactions in Health and Disease

Boehringer Ingelheim

Sophisticated cell-cell interactions between stromal and immune cells are known to be important across a range of biological processes most notably immune responses against pathogens, but also for productive tissue maintenance and repair. Despite the early experimental demonstration by Ludford In 1940 that leukocytes could significantly promote fibroblast proliferation *in vitro*[1], the importance of such interactions *in vivo* was not understood until the early 1970s. In a series of studies on herpes stromal keratitis, corneal inflammation and scar formation were associated with the pathological sequences of interactions between immune infiltrates and fibroblasts within the eye, which could be treated effectively with topical corticosteroids[2]. Since then and especially over the last two decades, there has been an explosive growth (**Figure 1**) in our understanding of the intricate and extensive cellular interactions between stromal cell types and tissue-resident or infiltrating immune cells. It is now well-appreciated that such interactions actively participate in a multitude of physiological and pathological processes ranging from successful acute wound healing to chronic disease conditions including autoimmunity, cancer, tissue fibrosis and infection.

The wide adoption of genetic engineering techniques in the late 1990's played an important role in demonstrating the complexity and importance of communication between stromal cells and immune cells *in vivo*. For example, by utilizing various transgenic and knockout mouse strains, it was shown convincingly that the formation and maintenance of secondary lymphoid organs (SLOs) such as the lymph nodes, spleen and peyer's patch required ligand-receptor engagement between lymphotoxin (LT) LTα1β2 produced by immune cells and LTβ receptor (LTβR) expressed on various stromal fibroblast subsets. LTβR –induced non-canonical NFκB activation in various stromal subsets within the SLOs is obligatory for the full differentiation of marginal reticular cells (MRC), follicular dendritic cells (FDC) and fibroblastic reticular cells (FRC). Conversely, stromal cell-derived chemokines such as CXCL13 govern the positioning of T and B cells, establishing SLO structures with segregated zones enriched with different immune cells. In addition, stromal cells within the SLOs can further control the development of innate lymphoid cellls (ILCs) and antigen specific regulatory T (Treg) cells, as well as the trafficking of effector T cells during immune responses[3].

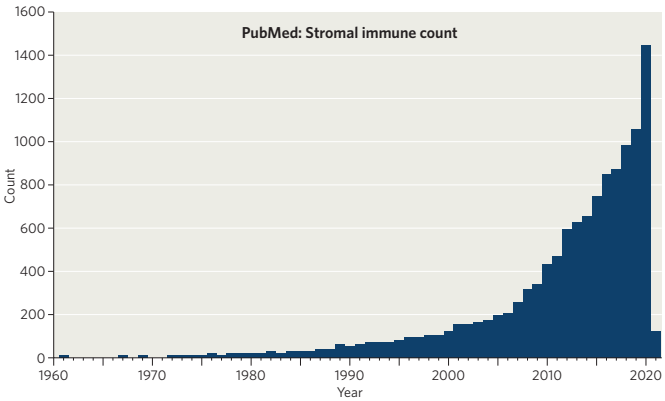

Figure 1. The explosive growth of biomedical literature studying the interactions between stromal and immune cells as shown by publications/year is based on PubMed data. In the past 20 years, the annual publications on this topic have increased by 10-fold, from ~100 in 2000 to well over 1000 in 2020.

The importance of stromal and immune crosstalk extends far beyond lymphoid organs and in fact governs many physiological processes besides immune responses. For example, successful pregnancy requires the establishment of an immune privileged maternal-fetal interface in which decidual stromal cells play an essential role; these stromal-driven processes include the production of IL-15 and m-CSF required for the respective differentiation of highly specialized decidual NK cells and macrophages with potent immunomodulatory activities, as well as limiting T cell infiltration through direct silencing of chemokine genes by stromal cell themselves[4].

Another well-studied example of stromal immune crosstalk *in vivo* is productive tissue repair such as acute wound healing following mild injuries, which requires a highly orchestrated process consisting of inflammatory, proliferative and remodeling/maturation phases[5]. In addition to the earlier demonstration that systemic depletion of macrophages resulted in reduced collagen formation and delayed re-epithelialization in tissue injury models, recent studies suggest that different macrophage subsets with inflammatory, reparative and resolving properties play dynamic roles during different phases of acute wound healing through their complex crosstalk

with stromal cells. For example, different macrophage subsets appear to provide differential cues controlling the proliferation and heterogeneity of fibroblasts/myofibroblasts during wound healing[6]. Conversely, mesenchymal stromal cells can instruct the adjacent macrophages to enhance phagocytic activity and increase amphiregulin production to facilitate the final phase of tissue remodeling and maturation[7], as well as provide chemotactic cues to distant monocytes through dynamic contraction[8].

Given the vital role of such cellular crosstalk interactions in regulating tissue homeostasis and repair, it had been speculated that dysregulated stromal-immune communication is a key pathogenic factor underlying many disease conditions[9]. Indeed, a number of human diseases are characterized by pathological tissue remodeling associated with dysregulated inflammatory and immune responses. These include inflammation-driven synovial fibroblast proliferation and pannus formation in the joints of rheumatoid arthritis patients, chronic inflammation and fibrosis in structuring and fistulizing Crohn's disease, and fibrotic granulomas in the lungs of patients with chronic Mycobacterium tuberculosis infection. In addition to being an important pathogenic mechanism, stromal-immune cell interaction can also influence the clinical response to immune-based therapeutics, as revealed by stromal remodeling following anti-PD1 treatment in cancer patients with melanoma, but only in those with durable responses[10]. Thus, targeting stromal-immune interactions may hold great promise as a potential therapeutic strategy in a wide variety of chronic diseases.

A major challenge facing such a therapeutic strategy rests with the complexity of stromal-immune cell interactions which may yield unanticipated competing beneficial and deleterious outcomes depending on the treatment context, including timing. For example, preclinical modeling with global macrophage depletion in the context of CCL4-induced liver fibrosis has shown that while depletion early of nearly all monocyte/macrophage populations in the injury phase can abrogate fibrosis (suggesting their critical role in fibrosis initiation), delayed depletion of all monocytes/macrophages during the recovery phase resulted in defective liver repair[11]. Similarly, depletion of all αSMA-expressing fibroblasts in a pancreatic cancer model unexpectedly resulted in Treg-mediated immunosuppression and accelerated pancreatic cancer with diminished survival[12]. These examples clearly highlight the critical need to identify and deeply understand the specific dysregulated stromal-immune interactions associated with different diseases, to advance selective targeting only of essential pathogenic driver interactions.

The tremendous technical advances in single cell RNA sequencing (scRNA-Seq) have revolutionized our ability to interrogate such complex cell-cell interactions, potentially allowing mechanistic delineation of such interactions. In a recently published study, scRNA-Seq of colon mucosa biopsies from healthy control and ulcerative colitis (UC) patients yielded >50 different cell populations including nine fibroblast subsets and eight macrophage subsets, of which the IL13RA2^{+}IL11^{+} inflammation-associated fibroblast (IAF) subset was uniquely associated with UC. Using ligand-receptor pairing analysis, the team was able to construct different stromal-macrophage interactions and even further isolate a potentially pathogenic cell-cell interaction associated with anti-TNF non-responsiveness, i.e. between OSM-producing inflammatory monocyte subset and OSMR-expressing IAF subset[13]. Similar approaches combining scRNA-Seq and ligand-receptor pairing prediction have now been successfully applied to other complex tissue and disease settings, uncovering potentially pathogenic immune-stromal cellular interactions in human colorectal adenocarcinoma[14], pulmonary fibrosis[15] and nonalcoholic steatohepatitis (NASH)[16].

At Boehringer Ingelheim, we are committed to the research and development of innovative drugs that significantly advance medical care in therapeutic areas such as oncology, cardiovascular and metabolic diseases, and in inflammatory and respiratory diseases. Our ambition is to bring the next generation of breakthrough therapies to patients, and we see the area of stroma cell-immune cell interactions as the next frontier of innovation. By sponsoring this collection, we wish to call attention to this emerging biology and its vast potential to govern both productive and pathological tissue responses in human health and disease. In doing so, we hope to further accelerate the science in this area, that in turn will yield insights that will lead to exciting new therapeutic opportunities for patients experiencing a broad range of currently poorly or untreatable debilitating and life-threatening conditions.

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