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CX3CR1 in Macrophage Efferocytosis and Its Impact on Thymic Immune Homeostasis

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Abstract. CX3CR1, the sole receptor of the CX3C chemokine family, exclusively engages CX3CL1 to direct immune cell trafficking and adhesion. Recent studies have further implicated CX3CR1 signalling in the modulation of intracellular Ca^{2+} levels. Efferocytosis —the rapid clearance of apoptotic cells by macrophages—depends on Ca^{2+}-dependent cytoskeletal rearrangements that drive targeting, internalisation, and degradation of apoptotic corpses. In the thymus, where the vast majority of developing T cells undergo apoptosis during positive and negative selection, efficient efferocytosis is essential for preserving the immune microenvironment and preventing autoimmunity. This review synthesizes current literature to investigate whether CX3CR1 fine-tunes macrophage efferocytic capacity via Ca^{2+} signalling and discusses the physiological significance of such regulation for thymic immune homeostasis. We conclude that the CX3CR1–Ca^{2+} axis represents a promising, yet under-explored, pathway linking chemokine signalling to central tolerance maintenance.

Keywords: CX3CR1, efferocytosis, thymus, apoptotic cells

1. Introduction

Massive apoptosis of thymocytes following negative selection is efficiently cleared via macrophage efferocytosis, a process essential for maintaining immune homeostasis in the thymus [1].

The chemokine CX3CL1, also known as fractalkine, is liberated from apoptotic cells after being cleaved by caspase-3. It functions as a "find-me" signal that binds to its receptor CX3CR1 on the surface of macrophages, thereby boosting macrophage chemotaxis and augmenting their phagocytic capacity [2]. CX3CR1 signaling further induces the upregulation of the bridging molecule MFG-E8, which in turn facilitates the recognition of "eat-me" signals. By inhibiting NF-κB and activating STAT3, this signaling pathway drives macrophages toward an anti-inflammatory, pro-resolving phenotype, thereby reinforcing the coupling between efferocytosis and anti-inflammatory responses [3]. Within the thymus, the receptor CX3CR1 is expressed by tissue-resident natural killer (NK) cells, macrophages, and dendritic cells. Impairment of the CX3CL1–CX3CR1 signaling axis has been linked to a wide range of pathological conditions, including liver fibrosis, malignancies, and pulmonary arterial hypertension [4]. Although this axis has been increasingly well characterized, its regulatory mechanisms and functional significance for thymic immune homeostasis—the thymus being the central organ of immune tolerance—have not been systematically reviewed. This review

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integrates recent in vivo and in vitro findings to delineate the functions of CX3CR1 and investigate how these functions modulate thymic macrophage activity and apoptotic cell clearance, thereby providing new insights into the regulation of autoimmunity and immune tolerance.

2. CX3CR1 and its functions

Chemokines form a family of small, soluble secreted proteins that regulate the trafficking and activation of immune cells through binding interactions with their corresponding specific chemokine receptors (CKRs) [5]. They are classified into four structural subfamilies—CC, CXC, CX3C, and XC—according to the number and spacing of conserved N-terminal cysteine residues [6]. Their receptors are seven-transmembrane domain proteins subdivided into classical G-protein-coupled CKRs and the atypical, scavenger-type chemokine receptors [7].

The human CX3CR1 gene spans 1,065 bp and encodes a 355-amino-acid polypeptide that contains the signature motifs of the chemokine-receptor superfamily. The CX3CL1–CX3CR1 dyad is a key regulator of inflammatory progression [8,9] and has also been identified as a driver of metabolic disorders, including chronic kidney disease, obesity, and atherosclerosis. CX3CR1 is expressed on monocytes, macrophages, dendritic cells, NK cells, specific subsets of T lymphocytes, and vascular smooth-muscle cells [10,11]. In mouse models of hepatic injury , the receptor constrains fibrogenesis by regulating the survival and differentiation of intrahepatic monocytes [12]; in the gut, it mitigates diet-induced dysbiosis, preserves intestinal epithelial barrier integrity, and attenuates steatosis, inflammation, and glucose intolerance in experimental models of non-alcoholic steatohepatitis [13]. Within the CNS, microglial CX3CR1 is highly active during early post-natal development, where it orchestrates synaptic surveillance and pruning. CX3CR1-deficient pups exhibit a transient reduction in microglial density, delayed synaptic pruning, accumulation of excessive dendritic spines, and persistently immature synapses—all of which correlate with measurable neurodevelopmental deficits [14,15].

3. Efferocytosis

Billions of apoptotic cells are generated daily in the human body, yet they are rarely detectable in histological specimens. This is because prompt phagocytic clearance prevents the leakage of harmful intracellular contents, thereby averting inflammation and tissue damage and preserving immune homeostasis [16,17].

Efferocytosis, the professional engulfment of apoptotic corpses, is distinguished by its remarkable capacity to eliminate large numerical burdens within defined temporal windows [18]. Both professional and non-professional phagocytes—including macrophages, DCs, epithelial cells, endothelial cells, and fibroblasts—participate in the recognition and phagocytosis of apoptotic bodies [19].

Macrophages integrate three hierarchically organized molecular cues—"find-me," "eat-me" and "don't-eat-me" signals—to ensure the precise targeting for apoptotic cells [20]. Operationally, efferocytosis proceeds through three sequential phases: recognition, internalization, and degradation.

Step 1: Apoptotic cells release soluble mediators—including nucleotides (ATP, ADP), lysophosphatidylcholine (LPC), sphingosine-1-phosphate (S1P), and the chemokine CX3CL1, among others—that act as chemotactic and priming factors to recruit phagocytes and upregulate their phagocytic and digestive machinery [21-23].

Step 2. Phosphatidylserine (PS) externalization on the outer layer of the plasma membrane in apoptotic cells serves as the primary, evolutionarily conserved "eat-me" signal, enabling apoptotic

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cells to bind tightly and with high affinity to the surface of phagocytes [24-26].

Step 3. The apoptotic corpse is subsequently internalized into a nascent phagosome, which fuses with lysosomes to form a phagolysosome. Within this phagolysosome, acid hydrolases mediate proteolytic and lipolytic degradation; recyclable metabolites such as putrescine and ATP are salvaged for reutilization [27,28].

Macrophages differentiate into two functionally opposing subsets [29,30]. The classically activated M1 phenotype is pro-inflammatory, secreting IL-1β, IL-6, IL-12, IL-23 and TNF-α, and consequently mediates antimicrobial immunity and inflammatory pathologies. The alternatively activated M2 phenotype is anti-inflammatory, characterized by abundant secretion of IL-10 and TGF-β to dampen immune responses; these cells predominate in helminth infection, asthma and allergic inflammation [31,32]. As pivotal innate immune effectors, macrophages constitute a frontline innate immune barrier and serve as the principal mediators of efferocytosis, leveraging their potent phagocytic machinery to preserve organismal homeostasis.

Efferocytosing macrophages exhibit a highly motile phenotype, continuously migrating via chemotaxis toward newly generated apoptotic corpses until intraphagosomal acidification and proteolytic catabolism are completed; this serial surveillance strategy secures rapid, high-capacity clearance [33]. Quantitative morphometry stratifies macrophages into low- (1–3), intermediate- (4–6), and high-burden (>7) subpopulations according to intracellular apoptotic body burden, whereas experimental paradigms that experimentally augment efferocytic cargo drive these cells toward an inflammation-compromised, functionally impaired state [34-37].

4. T-CELL development

The earliest T-lineage precursors originate from self-renewing hematopoietic stem cells (HSCs) in the bone marrow (BM), which give rise to multipotent myeloid and lymphoid progenitors. Multipotent lymphoid progenitors (MLPs) enter the peripheral circulation and extravasate into the thymus through a multistep adhesion cascade initiated by P-selectin glycoprotein ligand-1 (PSGL-1) on MLPs binding to constitutively expressed P-selectin on thymic microvascular endothelial cells [38]. Subsequent firm arrest and transmigration are mediated by the chemokine receptors CCR7 and CCR9, which sense CCL19/CCL21 and CCL25 gradients, respectively, released by thymic epithelial and stromal subsets [39-40]. Once within the thymic parenchyma, soluble and membrane-bound signals from the thymic microenvironment spatially orchestrate progenitor cell trafficking and stepwise differentiation [41,42].

At the cortico-medullary junction (CMJ), bone marrow (BM)-derived progenitor cells that migrate to this site come into contact with Delta-like ligand 4 (DLL4)—a Notch ligand—and the cytokine interleukin-7 (IL-7), both secreted by cortical thymic epithelial cells (cTECs). This signaling interaction drives early thymic progenitors (ETPs) to undergo irreversible differentiation and commit to the T-cell lineage [43,44]. Early thymic progenitors (ETPs) are characterized by a $CD4^{-}CD8^{-}$ phenotype, making them the most immature cell subset within the "double-negative" (DN) thymocyte population. Cells belonging to the DN subsets undergo stepwise rearrangement at the T-cell receptor (TCR) β gene locus; once functional V(D)J recombination takes place, the resulting TCR β chain can pair with the non-variable pre-Tα chain to assemble the pre-TCR complex. Signaling cascades triggered by the pre-TCR then prompt clonal proliferation, enforce allelic exclusion, and drive the differentiation of these cells into $CD4^{+}CD8^{+}$ "double-positive" (DP) thymocytes [45-47]. DP cells account for >80% of total thymocytes during early life and represent the primary target population for thymic selection.

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For DP thymocytes to pass positive selection, they are required to engage in moderate-avidity interactions with the self-peptide–MHC complexes displayed on the surface of cortical thymic epithelial cells (cTECs). The fate of lineage commitment is determined by the outcome of this interaction: thymocytes restricted to MHC-II molecules downregulate the expression of CD8, thereby developing into $CD4^+$ "single-positive" (SP) cells, while those restricted to MHC-I molecules turn off CD4 expression and differentiate into $CD8^+$ SP thymocytes [48]. SP cells then migrate toward the medulla, where they encounter thymic dendritic cells (DCs) and macrophages presenting a broader repertoire of self-antigens, including tissue-restricted antigens ectopically expressed under AIRE control. High-avidity recognition at this stage precipitates clonal deletion or diversion toward the Treg lineage, thereby establishing central tolerance [49]. Only ~5% of thymocytes successfully pass through these sequential selection checkpoints; the remaining 95% succumb to apoptosis and are swiftly removed by resident thymic macrophages and DCs via efferocytosis, preventing autoantigen release and maintaining intrathymic homeostasis [50].

5. Conclusion

By systematically reviewing the relevant literature on the function of CX3CR1, T cell apoptosis and autophagy, this research has identified that CX3CR1 may play a role in macrophage autophagy and is associated with Ca^{2+}. This not only provides novel references for the role of CX3CR1 in cell death, but also yields novel insights into the apoptosis and clearance of T cells in the central immune organ, the thymus. Furthermore, this pathway can serve as a therapeutic target for treating related diseases.

Existing literature has focused on CX3CR1-dependent cellular adhesion, directed migration and microbiota trafficking within the intestinal lamina propria; however, no systematic study has delineated the receptor's contribution to macrophage-mediated efferocytosis of apoptotic thymocytes, nor has the Ca^{2+}-dependent signaling axis recently suggested by single-cell transcriptomics been molecularly characterized. Age-associated thymic involution is accompanied by progressive parenchymal atrophy and concomitant accumulation of apoptotic debris, thereby elevating the metabolic and phagocytic burden on resident thymic macrophages to preserve immune homeostasis. Consequently, elucidating the mechanistic role of CX3CR1 in the clearance of dying thymocytes and its subsequent impact on thymic immune equilibrium constitutes a critical and under-explored direction for future translational investigation.

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