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Review

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Abstract

The controlled pro-inflammatory immune response is critical for fighting against external and endogenous threats, such as microbes/pathogens, allergens, xenobiotics, various antigens, and dying host cells and their mediators (DNA, RNA, and nuclear proteins) released into the circulation and cytosol (PAMPs, MAMPs, and DAMPs). Several pattern recognition receptors (PRRs) and their downstream adaptor molecules, expressed by innate and adaptive immune cells, are critical in generating the inflammatory immune response by recognizing PAMPs, MAMPs, and DAMPs. However, their dysregulation may predispose the host to develop inflammation-associated organ damage, neurodegeneration, autoimmunity, cancer, and even death due to the absence of the inflammation resolution phase. The cytosolic calcium (Ca^{2+}) level regulates the survival, proliferation, and immunological functions of immune cells. Cysteine-rich proteases, specifically calpains, are Ca^{2+}-dependent proteases that become activated during inflammatory conditions, playing a critical role in the inflammatory process and associated organ damage. Therefore, this article discusses the expression and function of calpains 1 and 2 (ubiquitous calpains) in various innate (epithelial, endothelial, dendritic, mast, and NK cells, as well as macrophages) and adaptive (T and B cells) immune cells, affecting inflammation and immune regulation. As inflammatory diseases are on the rise due to several factors, such as environment, lifestyle, and an aging population, we must not just investigate, but strive for a deeper understanding of the inflammation and immunoregulation under the calpain system (calpain 1 and 2 and their endogenous negative regulator calpastatin) lens, which is ubiquitous and senses cytosolic Ca^{2+} changes to impact immune response.

Keywords: calpains; inflammation; immunoregulation; adaptive immunity; innate immunity; immune cells

1. Introduction

Homeostasis defined as a critical process, which is not only governed by internal physiological and anatomical factors, such as hormones, neurotransmitters, enzymes, growth factors, and several other immune factors (cytokines, chemokines, and interferons or IFNs) but also external environmental factors, governing controlled functioning of various organs (lungs, liver, brain, kidneys, and heart etc.) that maintains an organism's well-being. Its failure often leads to the development of a disease, which, if not treated, may induce severe organ damage and eventually death of the person. Therefore, the association between disturbed homeostasis and disease outcomes and mortality underscores the urgency of understanding and maintaining homeostasis.

Immunological well-being, controlling an individual's homeostasis via maintaining the optimum immune function, is critically needed to fight against endogenous (dead cells and their extracellular components, such as DNA, mRNA, and proteins) and exogenous threats (pathogens, allergens, carcinogens, and traumatic injuries), called i**mmune homeostasis** or **immunohomeostasis** [1]. For example, the local tissue immune microenvironment comprises residential immune cells,

such as tissue-resident macrophages, dendritic cells (DCs), Langerhans's cells (LCs), innate-lymphoid cells (ILCs), and different types of T cells, including tissue-resident memory T cells (TRMs), which are guardians of organ homeostasis [2–4].

The dysregulated or derailed immune response is responsible for several pathologies, such as infectious diseases, allergies, cancers, neurodegenerative diseases, autoimmune diseases, autoinflammatory diseases, immunodeficiency diseases, and other inflammatory diseases, including metabolic disorders. Furthermore, teaching self-tolerance prevents the immune system from attacking itself, thereby maintaining homeostasis [5,6]. The events altering immune homeostasis may have a long-lasting impact through various mechanisms, including immune imprinting, reprogramming, reconfiguration, and remodeling, which can increase disease susceptibility [7].

The calpain system comprises two calcium (Ca^{2+})-dependent cysteine-rich ubiquitously expressed non-lysosomal cytosolic proteases called Calpain 1 (μ-calpain) and Calpain 2 (m-calpain) and one calpastatin polypeptide, which is a negative regulator of m-calpain [8,9]. Calpain 1 is referred to as μ-calpain because it requires a micromolar Ca^{2+} concentration for activation, whereas calpain-2 depends on a millimolar Ca^{2+} level; therefore, it is also referred to as m-calpain. The calpain-calpastatin interaction responsible for the negative regulation of two calpains has been discussed in detail elsewhere [8,10]. Calpains play a crucial role in cytosolic Ca^{2+}-dependent cellular functions, including cytoskeletal remodeling, cell cycle progression, gene expression, and cell death processes such as apoptosis and necroptosis, which are critical events in the pathogenesis of inflammation and immunoregulation [11–14]. In addition, calpain-mediated cleavage of cytoskeletal vimentin (a type III intermediate filament protein) during acute inflammatory conditions and infections in immune cells, such as macrophages induces their pyroptosis (an inflammatory cell death derived by membrane pore formation, inducing cell rupture due to osmotic influx), which further releases macro death/damage-associated molecular patterns (DAMPs, such as mitochondrial DNA and the mitochondrial cytochrome c oxidase 1 or Cox1) to aggravate the inflammatory process due to their immunostimulatory nature [15]. Hence, understanding inflammation and immunoregulation is critical, particularly through the lens of the calpain system. Therefore, the current article discusses the immunoregulatory and inflammatory effects of calpains, focusing on their expression and functions in primary immune cells, which are crucial for maintaining immune homeostasis.

2. Inflammation and Immune Dysregulation Are Keys to Disease Pathologies

Generating a pro-inflammatory immune response is a protective measure of the host against foreign molecules, such as pathogens and their components, called pathogen-associated molecular patterns (PAMPs) and/or microbe-associated molecular patterns (MAMPs), allergens, foreign antigens, xenobiotics, and endogenously induced damage/death-associated molecules (DAMPs) (Figure 1). These MAMPs, PAMPs, and DAMPs are recognized by several pattern recognition receptors (PRRs, such as absent in melanoma (AIM)-2-like receptors (ALRs), cytosolic guanosine monophosphate (GMP)-adenosine monophosphate (AMP) synthase (cGAS)-like receptors (cGLRs), toll-like receptors (TLRs), nucleotide-oligomerization domain (NOD)-like receptors (NLRs), C-type lectin receptors (CLR), RIG-like receptors (RLRs) and different scavenger and complement receptors acting as PRRs) expressed by innate and adaptive immune cells to neutralize/kill, and remove these threats from the system by generating a pro-inflammatory immune response, comprising release of different cytokines, chemokines, interferons (IFNs) and supporting antigen (Ag) presentation to adaptive immune cells (Figure 1) [16–29]. The mechanistic details of these PRRs expressed on different immune cells, in inflammation, inflammatory diseases, and immune regulation are discussed elsewhere [16,17,22–27,30–33].

If the immune system succeeds in removing the threat, it enters the resolution phase and then transitions into a state of homeostasis, known as immune homeostasis. (Figure 1) Notably, inflammatory immune response is a high cost and high benefit trait as it takes away the normal functioning of the organ and in some incidences it leads to permanent damage of the organ and even death of the person, on the other hand it saves the life by removing the external or endogenous threat

and even helps in the tissue or organ regeneration [34–37]. However, failure to generate a potent inflammatory immune response or to enter the resolution phase proves detrimental to the host, as both lead to the development of a chronic inflammatory stage, which is responsible for chronic lifestyle-based inflammatory diseases, cancer, autoinflammatory, and autoimmune diseases (Figure 1) [38,39]. Moreover, due to environmental and lifestyle changes globally, the incidence of six major immune-mediated inflammatory diseases (IMIDs): asthma, inflammatory bowel disease (IBD), multiple sclerosis (MS), rheumatoid arthritis (RA), psoriasis, and atopic dermatitis (AD), between 1990 and 2019 has increased [40].
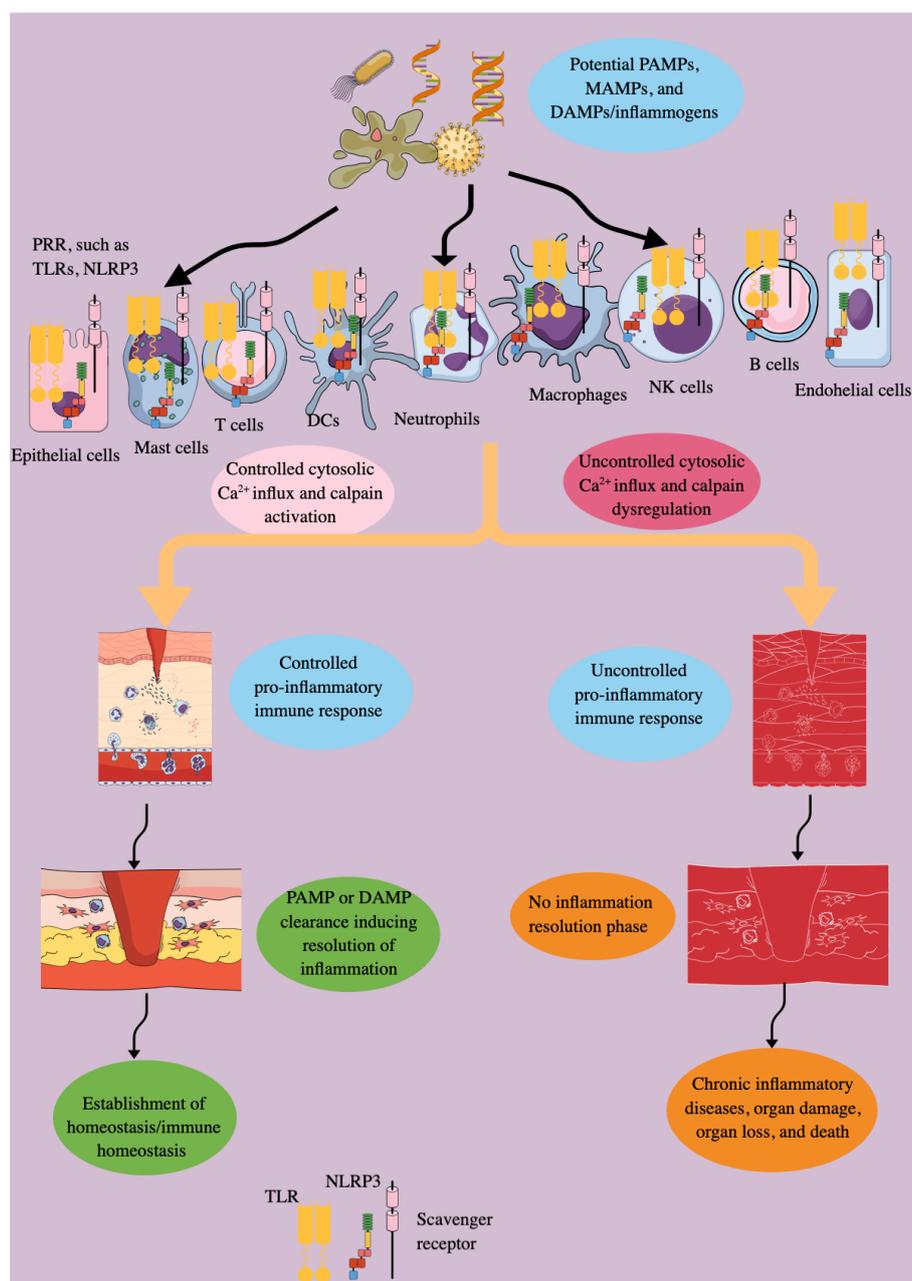
Figure 1. Schematic representation of protective regulatory inflammatory immune response and its dysregulation. In response to any exogenous or endogenous inflammogens, such as PAMPs, MAMPs, DAMPs, immune cells' PRRs activate downstream pro-inflammatory immune response that is dependent cytosolic Ca^{2+} and dependent proteases, such as calpains. The controlled activation of calpains helps to clear the potential threat via regulated immune response, which supports the establishment of tissue/organ homeostasis and immune homeostasis. On the other hand, dysregulated cytosolic Ca^{2+} and calpain activity aggravates inflammation and resolution phase is blocked, leading to chronic inflammation, organ damage, and in severe cases death.

The PRR activation exhibits a regulatory impact on cytosolic Ca^{2+} influx, and Ca^{2+} signaling also controls downstream signaling events mediated by their adaptor proteins, affecting pro-inflammatory and immunoregulatory activities depending on the immune cell type and the activated PRR (Figure 1) [41]. Therefore, along with other Ca^{2+}-dependent proteases, the expression and activity of calpains are impacted by the type of PRR activated in a specific immune cell type. For example, the activation of different TLRs and NLRP3 has been shown to impact calpain expression and activity in various inflammatory conditions and infectious diseases (Figure 1) [42–45]. Moreover, calpain 1 can cleave gasdermin D (GSDMD) during cell death, leading to pyroptosis, and the role of calpain in apoptosis is well established [46,47]. Pyroptosis is a critical type of programmed pro-inflammatory cell death that exacerbates inflammation, causing severe tissue/organ damage during various severe inflammatory and infectious diseases [48–51]. Hence, we must investigate inflammatory and immunoregulatory mechanisms through the lens of the calpain system, which are ubiquitous cytosolic cysteine proteases.

3. Calpain Expression and Their Actions in Different Immune (Innate and Adaptive) Cells

3.1. Epithelial Cells

are now considered potent innate immune cells due to expression of different PRRs, such as TLRs, NLRs, RLRs, and melanoma-differentiation-associated gene-5 (MDA-5), and they produce different innate immune mediators critical for antimicrobial, inflammatory, and immunoregulatory functions [52–60]. Even they interact with innate and adaptive immune cells to maintain immune homeostasis and also release several mediators, such as thymic stromal lymphopoietin (TSLP), IL-33, and B cell activating factor (BAFF), which modulate local accumulation and activation of Th2 responses and B cell immunoglobulin (Ig) production during different allergic and chronic inflammatory diseases [52,61–63]. Moreover, epithelial cells, such as nasal epithelial cells, primary airway epithelial cells (AECs), also generate type 1 (IFN-α and -β) and III (IFN-λ) interferons (IFNs) in response to microbial infections, such as severe acute respiratory syndrome-Corona virus-2 (SARS-CoV-2) and influenza A viruses (IAVs) [53,64]. TLR3 activation is critically involved in the production of type I and III IFNs by primary AECs via signal transducer and activator of transcription 1 (STAT1) activation [53]. At the same time, activation of pulmonary epithelial cells during *Mycobacterium tuberculosis*-induced airway infection/tuberculosis generates type II IFNs (IFN-γ) in a STAT1 activation-dependent manner to exert nitric oxide (NO·)-dependent mycobactericidal effects [65].

Additionally, AECs also express and secrete various complement proteins, including complement component 3 (C3), C5, and factor B, which are crucial for their complement-associated immunoregulatory effects and survival during acute infections or sterile inflammation-associated insults [66–69]. For example, endogenous C3 of AECs and their tendency to load exogenous $C3H_2O$ rescues them from cell death induced by several factors, such as hydrogen peroxide (H_2O_2) and growth factor deprivation [66]. However, C3 mitigates AEC death in some conditions, which are independent of apoptosis and pyroptosis [66]. These AECs secrete C3 constitutively, which further increases during infectious or inflammatory conditions. For example, AEC C3 protects against pneumonia-associated acute lung injury (ALI), a condition that also requires the alternative complement component factor B [70]. Thus, skin epithelial cells and epithelial cells at mucosal surfaces are potent innate immune cells that create a borderline defense between the host and the external environment, which further signals adjacent or local and distant immune cells via direct interaction or the secretion of immune mediators.

Calpain 1&2 are expressed in different epithelial cells, such as respiratory, gastrointestinal, urinary tract, and reproductive tract, including breast tissue environment, and human lens epithelial cells (LECs), where they play critical role in epithelial cells' immune-modulatory functions and death under different inflammatory insults, including infectious diseases, ischemia, and cataract (Figure 2A) [8,71–73]. Moreover, a significant increase in calpains expression and activity due to increased

release of Ca^{2+} from endoplasmic reticulum (ER) in LECs of patients with diabetic cataract has been observed, which is directly associated with apoptotic death of LECs via their structural proteins, such as vimentin and supporting transformation of soluble crystallin into insoluble truncated form, which scatters light (Figure 2A) [74–77]. Recent data has suggested the involvement of the immune system in cataract pathogenesis, as calpastatin level in human LECs decreases with aging causing overactivation of calpains, which might induce the release of pro-inflammatory cytokines, such as IL-1α and acquire the senescence-associated secretory phenotype (SASP), activating local innate immune cells and recruiting distant immune cells to create a tissue destructive pro-inflammatory environment [72,78]. Moreover, calpain activation induces their death, which further stimulates pro-inflammatory immune response. Thus, calpain activation in LECs within the immune-privileged environment of the lens triggers a pro-inflammatory response, potentially aggravating or initiating the pro-inflammatory events associated with age-related cataract, which has a significant correlation with the activation of pro-inflammatory immune responses (Figure 2A).
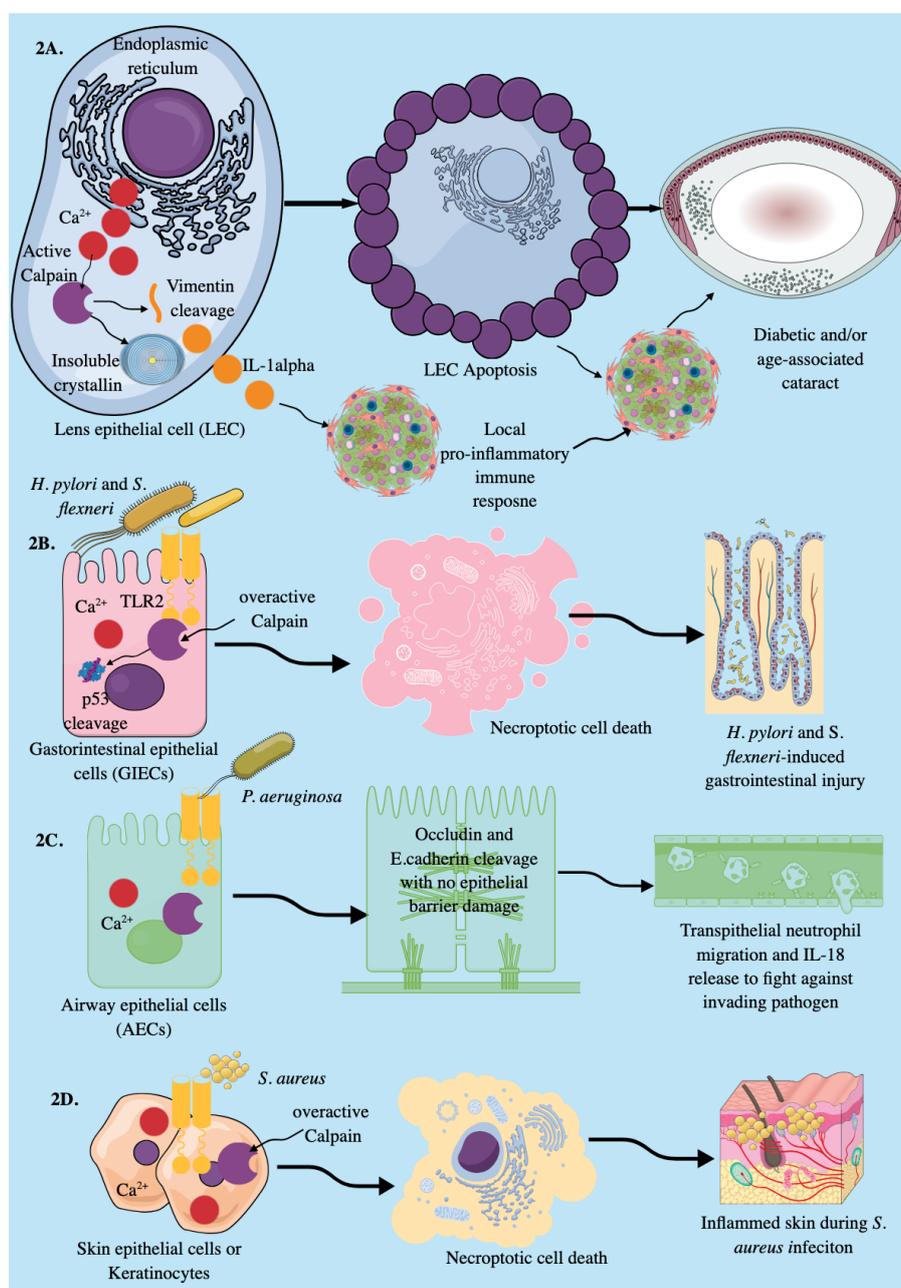

Figure 2. Calpains in different epithelial cells and their role in their pro-inflammatory action. **2A.** Calpain activation in LECs during diabetic or age-associated cataract induces cytosolic Ca^{2+}-dependent calpain

activation, which cleaves cytoskeletal vimentin protein, induces insoluble crystallin, mature IL-1α release, inducing local pro-inflammatory immune response, and LEC apoptosis. **2B.** Gastrointestinal epithelial cells during infections caused by *H. pylori* and *S. flexneri* induce their necroptosis via TLR2 dependent calpain activation, which cleaves p53 to prevent their apoptosis. This causes *H. pylori* and *S. flexneri*-induced gastric ulcers and intestinal injury. **2C.** AECs during *P. aeruginosa* lung infection induce transepithelial migration of neutrophils to contain the infection without damaging epithelial barrier in a calpain activation-dependent manner via TLR2 signaling pathway. **2D.** Skin epithelial cells or keratinocytes during *S. aureus* infection via TLR2 signaling-dependent cytosolic Ca²⁺ influx activates calpains, which induces their necroptosis, which aggravates infection and associated inflammation.

Signaling Events Inducing Calpain Activation in Epithelial Cells to Induce Their Immunological Functions

TLRs are critical PRRs that regulate innate and adaptive immune responses during various infectious and inflammatory conditions to maintain immune homeostasis [1,16,18,21,79,80]. Epithelial cells at various mucosal surfaces express different TLRs, and the interaction between gut microbiota and gut epithelial TLRs is critically needed to maintain gut homeostasis, including gut immune homeostasis [81–83]. The loss of this equilibrated gut epithelial TLRs-microbiota interaction may cause inefficient clearance of pathobionts, which may increase the incidence of gastric infections due to disturbed immune homeostasis and microbiota. Critical illness, such as sepsis may also cause gut failure leading to the disturbed gut epithelial TLR-microbiota interaction, which may long lasting impact on local (gut) and systemic immunity [84]. Moreover, this prolonged alteration in gut microbiota may further disrupt this homeostatic interaction, increasing the chances of developing colitis, inflammatory bowel disease (IBD), and colorectal cancer (CRC) [81]. Similar to the gut epithelial-microbiota interaction via TLRs, pulmonary epithelial cells (PECs), such as those in the nasal, tracheal, and bronchial epithelia, which express different TLRs, interact with their local microbiota to maintain local and systemic immune homeostasis [53,85–87]. The nasal epithelial cells and primary airway epithelial cells (AECs) highly express TLR3, 7, and 9, which enable them to sense pathogenic viruses and mount an initial innate immune response to maintain local immune homeostasis [86]. However, an altered lung microbiome, like an altered gut microbiome, is also associated with chronic pulmonary inflammatory diseases, such as allergic asthma, chronic obstructive pulmonary disease (COPD), frequent episodes of pneumonia, and even lung cancer [88]. Skin epithelial cells, such as keratinocytes, also express various TLRs, which interact with the local microbiota to maintain a healthy skin microbiome, homeostasis, and immune homeostasis [89–91]. Altered skin microbiota is associated with various chronic inflammatory skin diseases, including acne vulgaris and atopic dermatitis (AD) [92] Thus, epithelial cell TLRs play a critical role in maintaining a healthy local microbiota (gut, lungs, and skin) and immune homeostasis.

The activity of cytosolic calpains depends on the intracellular Ca²⁺ levels. TLR signaling has been shown to increase cytosolic Ca²⁺ levels by interacting with Ca²⁺ sensing proteins, such as STIM1 (Stromal interaction molecule 1), which controls Ca²⁺ flow into the cell by activating different Ca2+-release-activated channels (CRACs), such as Orai1 [93–95]. Knockdown of these CRACs or chelation of cytosolic Ca²⁺ decreases the TLRs (TLR3 and TLR4) stimulation-induced TNF-α and IL-6 release by innate immune cells, such as astrocytes, by decreasing cytosolic Ca²⁺ levels by regulating store-operated Ca²⁺ entry (SOCE) pathways [94,95]. LPS-stimulated CD14-dependent intracellular Ca²⁺ fluxes also induce TLR4 internalization/endocytosis, a critical step for downstream TRIF-related adaptor molecule (TRAM) and Toll/IL-1 receptor (TIR) domain-containing adaptor-inducing IFN-β (TRIF)-dependent signaling, which promotes the expression of interferons (IFNs) and IFN signaling genes (ISGs) [96–98]. The endosomal TLR4, which contains a TIR domain, interacts with phospholipase Cg2 (PLCg2), spleen tyrosine kinase (SYK), and E1/E2 ubiquitination and undergoes degradation as it lacks canonical TLR signaling adaptor molecules and cascades [99]. Endosomal TLR2 signaling via TRAM is also critical for the type 1 IFN response during herpes simplex virus (HSV) and *Staphylococcus aureus* (*S. aureus*) infection, facilitating the clearance of the infection [100].

Moreover, TLR9 signaling is also significantly attenuated (decreased IL-1α and IL-1β secretion) in the presence of Ca^{2+} chelators (EGTA-AM) and calcineurin inhibitor (FK-506) due to the inhibition of IκBβ (a NF-κB inhibitory protein) degradation [101]. Thus, TLR signaling increases cytosolic Ca^{2+} levels, which are crucial for maintaining normal immune homeostasis during host-microbiota interactions in various compartments and in response to infectious and inflammatory conditions, thereby enabling the defense against diverse pathogens and inflammatory agents.

TLR2 signaling in different epithelial cells, such as gastric, intestinal, skin, and urinary tract epithelial cells, activates calpain activity in response to different pathogens, such as *Helicobacter pylori* (*H. pylori*), *Staphylococcus aureus* (*S. aureus*), *Chlamydia trachomatosis* (*C. trachomatosis*), and *Neisseria gonorrhoeae* (*N. gonorrhoeae*) (Figure 2B-2D) [42,102]. The activation of calpains in human gastric epithelium in response to chronic TLR2 stimulation promotes inflammatory tissue damage and disrupts adherens junctions, as seen during *H. pylori* infection, which is a critical risk factor for gastritis, gastroduodenal ulcers, and gastric adenocarcinoma induction and development (Figure 2B) [102]. Moreover, patients with *H. pylori* infection exhibit elevated circulating 80-kDa E-cadherin ectodomain. The TLR2 inhibition not only blocked calpain activity but also adherent junction disassembly. Similarly, *Shigella flexneri* (*S. flexneri*) infection, via TLR4 and TLR2 activation in intestinal epithelial cells (IECs), activates calpains by increasing cytosolic Ca^{2+} s a result of genotoxic stress induction and its virulence factor, VirA (Figure 2B) [42,103].

The activated calpains cleave p53 (critical for DNA repair) to prevent its apoptotic death, thereby supporting the pathogen's growth and multiplication inside the infected IECs, which later induces necroptosis and exaggerated inflammatory tissue damage (Figure 2B) [42,103]. Moreover, IEC Shigella infection alters its sumoylation process by activating calpains, which inhibit the SUMO E1 enzyme SAE2, thereby supporting its entry into IECs by limiting cytoskeletal rearrangements induced by bacterial effectors [104]. Sumoylation plays a critical role in host defense during *S. flexneri* infection by regulating intestinal permeability and restricting epithelial invasion, thereby controlling mucosal inflammation [105,106]. Thus, IEC calpain activation during *S. flexneri* infection not only expedites the IEC infection process by modifying cellular architecture through the cleavage of different cell membrane proteins but also prevents their apoptosis via p53, supporting their intracellular growth and multiplication (Figure 2B). However, TLRs (TLR2 and TLR4)-dependent local macrophage activation during shigellosis produces IL-12, which induces IFN-γ production from local innate lymphocytes (NK and $\gamma\delta$T cells) and controls *S. flexneri* replication in IECs [107,108]. IEC can also produce IFN-γ, which may protect intestinal epithelial barrier integrity by inducing the production of IL-18 binding protein (IL-18bp), comprising the IL-18/IL-18bp system (an anti-inflammatory system) and inducing their early apoptosis to prevent excess pathogen growth and inflammatory tissue damage [109,110]. Thus, it will be interesting to observe the impact of calpain activation on IFN-γ production during epithelial cell infections, such as *those caused by S. flexneri*. The selective protein targets of calpains, critical for cell motility and adherence, have been discussed in detail elsewhere [111–113].

On the other hand, in the pulmonary lung microenvironment, TLR2 stimulation-mediated calpain activation in PECs during *Pseudomonas aeruginosa* (*P. aeruginosa*) infection cleaves occludin and E-cadherin (transmembrane junctional proteins) without disturbing the epithelial barrier integrity (Figure 2C) [44,114]. The calpain-mediated cleavage of occludin and E-cadherin is critical for the transepithelial migration of neutrophils to combat invading respiratory pathogens, along with the release of chemokines, such as IL-8 (Figure 2C). Hence, TLR2 signaling-induced cytosolic Ca^{2+} upregulation activates calpains in mucosal epithelial cells, which facilitates the transepithelial migration of potent innate immune cells, such as neutrophils, to combat invading pathogens and control mucosal inflammation by regulating epithelial barrier functions (Figure 2C) [114,115]. Moreover, in calpain 1 and 2 knockout mice subjected to acute bacterial peritonitis, decreased neutrophil infiltration and associated bacterial clearance have been observed, indicating a critical role of calpains in neutrophil infiltration at the site of infection [43]. The peritoneum is a simple squamous cell epithelial lining of the abdominal cavity. These studies further indicate that the activation of

calpain in epithelial cells and myeloid immune cells (MICs) during bacterial infections is critical for clearing the infection and maintaining immune homeostasis.

The signal strength and duration of TLR signaling in epithelial cells are critical determinants of calpain-mediated tissue-damaging effects, such as epithelial barrier damage resulting from cell death, as observed during *H. pylori* infection. Moreover, during *S. aureus* skin infection, keratinocytes die due to their necroptosis in response to overactivated calpains in response to TLR2 signaling and calpastatin (a natural calpain inhibitor) inhibition (Figure 2D) [42,116]. Furthermore, calpain activity is critical for clearing the infection during acute bacterial peritonitis, and its deficiency leads to the development of bacteremia that can progress into sepsis if the infection has not been cleared [43]. Hence, calpains are critical for epithelial cell-mediated immunoregulatory functions and inflammatory diseases.

3.2. Endothelial Cells

Endothelial cells are also considered as innate immune cells due to expression of different active PRRs, antigen presentation, phagocytosis, and secretion of several inflammation mediators, such as cytokines, chemokines, and type 1 IFNs [16,117–122]. Endothelial cells also express calpains, which are crucial for their angiogenic function and inflammatory response upon exposure to inflammatory stimuli, inducing changes in their expression and activity that regulate transcellular permeability (Figure 3A) [123,124]. For example, vascular endothelial growth factor-2 (VEGF-2) activates calpain-2 (m-calpain) to promote angiogenesis under normal conditions without involving calpain 1 or μ-calpain by activating phosphatidylinositol 3 kinase (PI3K)/5'-Adenosine monophosphate (AMP) activated protein kinase (AMPK)/Akt-dependent endothelial nitric oxide synthase (eNOS) phosphorylation and nitric oxide (NO.) production in endothelial cells (Figure 3A) [123,125–127]. This VEGF-dependent angiogenic effect on endothelial cells is regulated by a calpain-dependent negative feedback loop that inhibits overactivation of VEGF receptor 2 (VEGFR2) (Figure 3A) [128]. Calpain cleaves and activates protein tyrosine phosphatase type 1B (PTP1B), which dephosphorylates VEGFR2 (Figure 3A) [128].

Failure of this calpain-dependent negative feedback (PTP1B inhibition or overexpressed/overactive calpains) is seen in impaired wound healing in experimental animals with diabetes developing diabetic wounds (Figure 3A) [128]. Moreover, diabetes-associated high glucose concentrations, or hyperglycemia, downregulate focal adhesion kinase (FAK) expression by inducing calpain-1 overexpression, which cleaves FAK explicitly and is responsible for the abnormal architecture of healed diabetic ulcers and recurrence [129]. However, under non-diabetic conditions, the targeted deletion of endothelial calpains (CAPNS1, the common regulatory subunit of Calpain-1 and 2) decreases the wound healing process by reducing their inflammatory functions, such as the generation of NF-κB-dependent pro-inflammatory cytokines (TNF-α, critical for endothelial cell proliferation, migration, and tube formation) and downregulating β-catenin expression (Figure 3A) [130]. Thus, in diabetic wounds, overactive calpains impair the wound-healing process, whereas calpain inhibition impairs the normal wound-healing process (Figure 3)A.

IFN-induced protein 10 (IP-10) or CXC motif chemokine ligand 10 (CXCL10) via binding to CXCR3 inhibits VEGF-induced endothelial tube formation and motility by inhibiting calpain activity via upregulating cAMP and protein kinase A (PKA) activity [126]. CXCL10 levels have been found to increase in healing and non-infected diabetic wounds, which, by inhibiting calpains, may induce impaired wound healing [131]. In addition, CXCL10 is associated with the resolution of active proliferative diabetic retinopathy and the development of traction diabetic retinopathy, which correlates well with VEGF levels and activity on retinal endothelial cells [132]. Thus, it is interesting to study the CXCR3/CXCL10/calpain axis in the context of VEGF in patients with diabetic wounds and retinopathy, which lack proper healing and re-emerge after healing. On the other hand, lower circulating levels of CXCL10 are associated with diabetic kidney disease, characterized by excess fibrosis, which may be due to increased calpain activity [133]. Furthermore, calpain inhibition has shown beneficial effects in a swine model of myocardial fibrosis in chronic ischemic

hypercholesterolemia [134]. Thus, understanding endothelial calpain regulation is a critical factor in combating various inflammatory and wound healing issues associated with diabetes and other chronic inflammatory diseases, such as chronic artery disease.
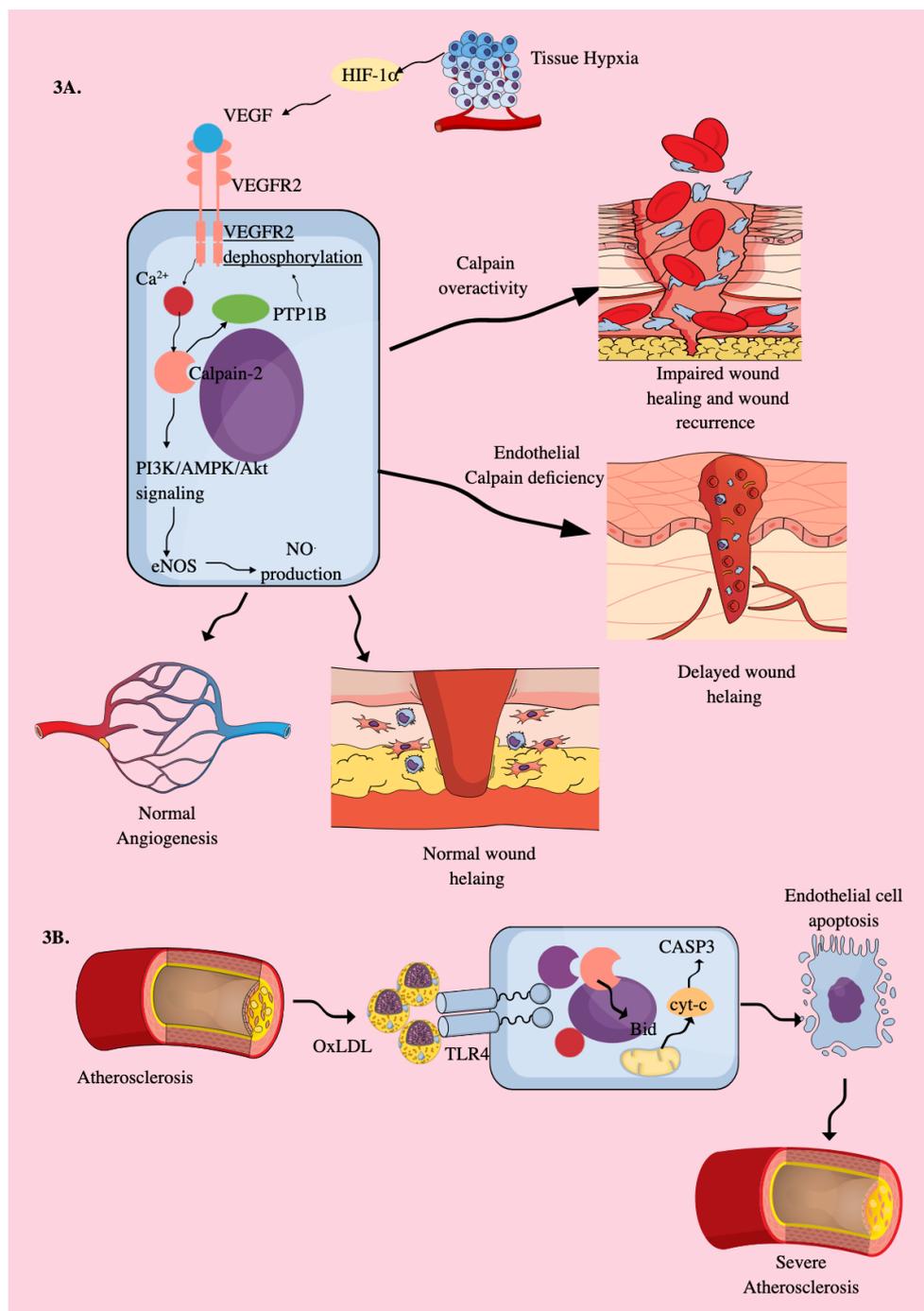

Figure 3. Endothelial calpains in their physiological and immunological functions. **3A.** Under normal conditions, endothelial calpains in response to VEGF via VEGFR2 interaction induce calpain activity, which via PI3K/AMPK/Akt signaling induces eNOS activation. The activated eNOS generates NO·, which is critical for angiogenesis and wound healing. However, overactivated calpain activity in diabetic patients impairs their wound healing process and even healed wound recur. Moreover, endothelial cell calpain deficiency delays wound healing. **3B.** In atherosclerosis, oxLDLs, activate TLR4, which via calpain activation cleaves Bid that induces cytochrome c (cyt-c) release from the mitochondria. Cyt-c induces CASP3 activation and endothelial cell apoptosis, which increases the atherosclerosis severity.

VEGF also downregulates calpastatin (an endogenous calpain inhibitor that prevents the induction of pathogenic angiogenesis) expression in endothelial cells, which is observed during chronic inflammatory conditions, including cancers that exhibit altered angiogenesis or neoangiogenesis in response to pro-inflammatory cytokines, such as IL-6 and VEGF [135]. Endothelial calpastatin, by inhibiting calpains prevents suppressor of cytokine signaling 3 (SOCS3) molecule's degradation that keeps in check the IL-6/STAT3/VEGF-C axis-mediated neoangiogenesis in inflammatory hypoxic environment seen in tumor microenvironment (TME), diabetic retinopathy, and non-healing wounds [135–137].

In addition to VEGF-1, fibroblast growth factor (FGF) and epidermal growth factor (EGF) also activate calpain activity in endothelial cells [123]. Hypoxia also elevates calpain expression and activity in endothelial cells, which inhibits the NF-κB inhibitory activity of IκB and promotes inflammatory events in these cells, a process essential for normal wound healing, as discussed earlier (Figure 3A) [130,138,139]. Furthermore, hypoxia via hypoxia-inducible factor-1α (HIF-1α) also elevates VEGF expression and Na$^+$/H$^+$ exchanger-1 (NHE1) expression, which further activates calpain expression and function under chronic hypoxic conditions (Figure 3A) [140]. Calpain-1 mediates HIF-1α expression during hypoxia through NF-κB (P65) activity [45].

Atherosclerosis is a chronic inflammatory condition that affects the vascular endothelium. Obesity, high-fat diet (HFD), and cigarette/tobacco smoking are critical factors for atherosclerosis development. Patients with atherosclerosis or the aforementioned lifestyles have high circulating oxidized low-density lipoproteins (OxLDLs), which endothelial TLR4 recognizes to initiate an inflammatory cascade, including increases in intracellular Ca^{2+} and dependent calpain activity, as well as endothelial cell death, and an increase in atherosclerosis severity (Figure 3B) [16,141–146]. OxLDL-induced endothelial cell death involves calpain-dependent Bid cleavage and subsequent cytochrome-C (cyt-c) release from the mitochondria, which activates caspase 3 (CASP3) (Figure 3B) [143]. OxLDL also induces pathogenic changes in the metabolism, transcriptome, and epigenome of endothelial cells before the induction of a typical inflammatory endothelial phenotype through AP-1, NFE-2, and CEBP transcription factors [147]. In addition to vascular endothelium, lymphatic endothelial cells also exhibit calpain dysregulation during hypercholesterolemia in response to lysophosphatidic acid, which limits their ability to stabilize regulatory T cells (Tregs) and further supports inflammation [148]. The overexpressed and overactive calpain in lymphatic endothelial cells cleaves mitogen-activated protein kinase kinase kinase 1 (MEKK1) and subsequently cleaves its downstream target, TGF-β1. The inhibition of calpain in lymphatic endothelial cells inhibited inflammatory atherosclerotic plaque formation and increased Tregs in the peripheral circulation, which further reduced aortic atherosclerotic plaque formation in mice with hypercholesterolemia [148]. Moreover, lysophosphatidic acid-mediated overactive calpains increase the IL-18/NF-κB/vascular cell adhesion molecule 1 (VCAM1) axis in lymphatic endothelial cells, thereby inhibiting lymphocyte mobility on the cells [148]. Calpain 1 deletion attenuates atherosclerotic plaque formation and improves vasomotor dysfunction in apolipoprotein E1 (ApoE1) knockout mice [149].

In genetically modified mice (endothelial-specific Capn4 knockout (TEK/Capn4$^{-/-}$), LPS-induced acute endotoxemia did not develop severe acute kidney injury (AKI) as shown by wild-type (WT) mice [150]. Capn4 deletion in vascular endothelial cells protected them from AKI by preventing their apoptotic death, decreasing systemic and renal tissue reactive oxygen species (ROS) and NO. levels/production due to suppression of inducible and endothelial NOS (iNOS and eNOS) in kidney tissue [150]. The decreased iNOS activity was associated with lower calpain and associated p38MAPK activities in vascular endothelial cells. Moreover, traumatic brain injury (TBI)-induced hyperpermeability of the blood–brain barrier (BBB) also involves an overactive endothelial calpain system and can be reversed by inhibiting overactive calpain activity [151]. In addition to sterile inflammatory conditions, the endothelial cells' infections seen during echovirus 1 (E1), Coxackievirus B3 (CVB3), flaviviruses, and chikungunya virus activate calpain to support their entry, replication, endothelial cell polarization, and their death, which may cause vascular leakage in severe cases causing severe damage and patient's death [42,152–155]. Thus, abrogated calpain activation in

endothelial cells during severe inflammatory conditions, such as sepsis, induces their apoptotic death, breaching the endothelial barrier, resulting in endothelial vascular leakage (increased inflammatory immune cell transendothelial migration, protein and fluid leakage), causing edema, organ damage, and death.

However, controlled vascular endothelial calpain activity is critical for diapedesis to fight against invading pathogens and inflammatory tissue insults, as calpain inhibition blocks transendothelial lymphocyte migration or diapedesis due to impaired development of intercellular adhesion molecule 1 (ICAM-1)-rich docking structures by the endothelial cell [156–158]. Moreover, endothelial cells defective in myeloperoxidase (MPO) production show defective calpain activity, eNOS production, and the VCAM-1 expression, which is critical for diapedesis [157,159]. Hence, regulated calpain activity in endothelial cells is critical for the transendothelial migration of lymphocytes at the site of infection and inflammation, thereby maintaining homeostasis or immune homeostasis. However, endothelial cell overactivation of calpain may cause vascular leak, leading to exaggerated inflammation and organ damage, as seen in sepsis.

3.3. Calpains in Myeloid Innate Immune Cells (MICs)

Macrophages, neutrophils, dendritic cells (DCs), and mast cells are critical myeloid innate immune cells with pro-inflammatory and immunoregulatory functions [17,160–162]. All these MICs express conventional calpains (calpain 1 and 2) and regulate their pro-inflammatory and immunoregulatory functions under homeostasis and different pathologies [163].

3.3.1. Macrophages:

During homeostasis or normal conditions, cytosolic calpains, which are mainly attached to endoplasmic reticulum (ER) membranes, specifically calpain 2, cleave selenoprotein K (SelK), which is also attached to ER membranes close to the operating calpain/calpastatin system [164]. The calpain-2 cleaves SelK into a truncated SelK form lacking a selenocysteine residue in resting or non-stimulated macrophages. Additionally, another study has indicated the presence of full-length SelK in calpain 1 and 2 KO naïve macrophages [43]. However, in macrophages stimulated with different TLRs, such as TLR2, TLR3, TLR4, and TLR9, the increased calpastatin activity blocked the calpain protease activity responsible for cleaving the SelK [164]. The full-length SelK is required for Ca^{2+} flux and migration of macrophages at the site of inflammation in response to chemoattractants, such as monocyte chemoattractant protein-1 (MCP-1) [164]. Moreover, calpain KO murine macrophages (including bone marrow-derived macrophages (BMDMs) and peritoneal macrophages) exhibit defective phagocytosis, characterized by decreased intracellular killing (ICK) of engulfed bacteria (Figure 4A) [43]. The decreased ICK may be associated with reduced intracellular reactive oxygen species (ROS) generation in macrophages as seen in calpain KO neutrophils, which fail to kill enterobacteria *in vivo* in a mouse model of acute bacterial peritonitis [43]. However, in a pulmonary inflammatory environment, such as cystic fibrosis (CF), neutrophil elastase increases cytosolic Ca^{2+} and calpain-2 activation in murine alveolar macrophages and human monocyte-derived macrophages (hmDMs) from patients with CF, which impairs their phagocytic potential by cleaving cytoskeletal proteins (Talin and Ezrin) (Figure 4A) [165]. Moreover, neutrophil elastase also decreases calpastatin activity in murine alveolar macrophages isolated from mice with CF and hMDMs of patients with CF, which further increases calpain-2 activity. Interestingly, no differences in Calpain activity, Calpain-2 and Calpastatin expression, and intracellular calcium between CF and non-CF macrophages have been reported at baseline level. Thus, animal species, disease specificity, and the location of macrophages (organ-specificity), along with external stimuli, play a critical role in calpain-mediated immune/inflammatory functions of macrophages, which must be studied [166–169]. For example, macrophages isolated from patients with CF exhibit abnormal expression and trafficking of TLR4, decreased expression of HLA-DR and HLA-DQ (MHC-II molecules that present antigens to CD4+T cells), and a decreased phagocytic potential compared to those of normal humans[170–173].
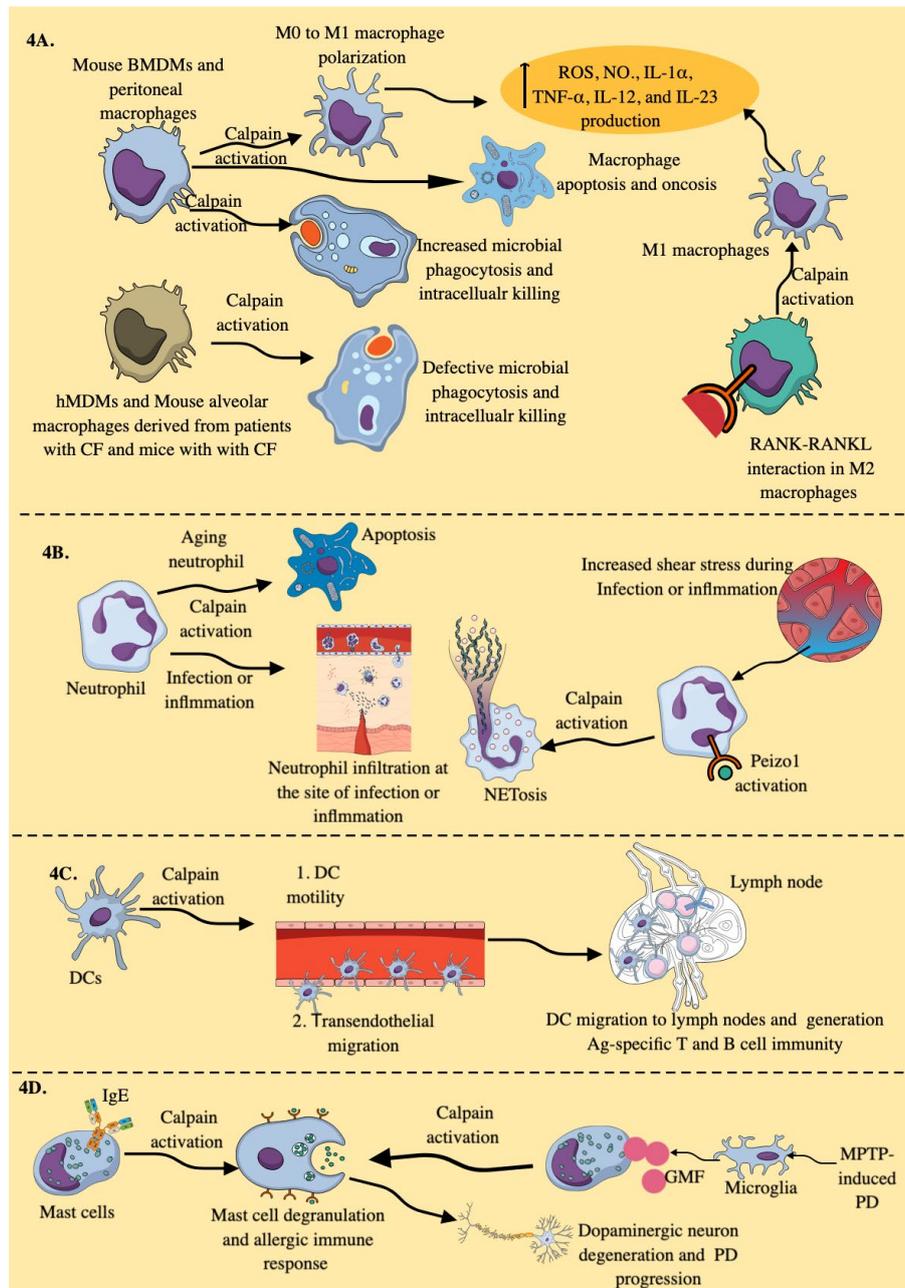
Figure 4. Calpain in macrophages. In mouse BMDMs and peritoneal macrophages, during bacterial infections (please see the text) calpain activation is critical for M0 to M1 macrophage polarization, phagocytic killing of the phagocytosed bacteria, macrophage apoptosis and oncosis, and release of several pro-inflammatory mediators. RANK-RANKL interaction in M2 macrophages during infection and in TME polarizes M2 macrophages to M1 macrophages via calpain activation. On the other hand, hMDMs and murine pulmonary macrophages isolated from mice and patients with CF calpain activation in associated with their defective phagocytic activity. **4B.** Under normal condition, aging neutrophil overexpress calpains, which are critical for their apoptosis, but during infection or inflammation neutrophil calpain activation is critical for their transendothelial migration/diapedesis. Moreover, neutrophil calpain activation in response to shear stress and piezo 1 activation induces NETosis, which is critical in inflammation and immune homeostasis maintenance. **4C.** Calpain activation in DCs is critical for their motility, transendothelial migration to reach to lymph nodes during infection or in response to vaccination to generate Ag-specific T and B cell-dependent immunity. **4D.** Calpain activation in mast cells in response to IgE-mediated immune response induces mast cell degranulation and supports NF-κB-dependent pro-inflammatory action. Moreover, in MPTP-induced mouse model of PD, microglia-derived GMF activates mast cells, which via calpain activation induces their degranulation and dopaminergic neuron degeneration that helps in PD progression. Kindly see the text for detail.

Moreover, several other bacterial infections caused by *Streptococcus agalactiae* (a Group B streptococcus or GBS), *S. pyogenes* (a Group A streptococcus), *Bacillus anthracis*, *Listeria monocytogenes*, *Klebsiella pneumoniae*, and *Mycobacterium tuberculosis* utilize the calpain system for their survival, growth, and multiplication by inducing their cytoskeletal derangements, decreasing phagosome and phagolysosome formation, and apoptosis [42,174]. For example, *S. agalactiae* and *S. pyogenes* infections induce apoptosis and oncosis (a form of pro-inflammatory programmed cell death characterized by cell swelling and vacuolization of the cell cytoplasm, causing plasma membrane rupture) in macrophages by increasing calpain activity (Figure 4A) [42,175–177]. The detailed mechanisms of macrophage apoptosis, including the role of calpains during GBS infection, have been discussed elsewhere [42,178]. Moreover, GBS activates the PI3K/AKT signaling pathway in macrophages, which also involves the calpain system for their intracellular survival [179]. *L. monocytogenes* in macrophages via listeriolysin O (LLO) utilizes the calpain system by increasing cytosolic Ca^{2+} levels to escape from phagosome maturation for their intracellular growth and multiplication [180,181]. Even *L. monocytogenes* utilizes calpain 2 to evade the intestinal immune barrier, such as Peyer's patches (PPs), via iNOS-induced NO·, which, along with other critical immune cells, also includes macrophages [182–184]. Moreover, different *M. tuberculosis* strains also induce calpain overactivation in macrophages by increasing cytosolic Ca^{2+} levels, which facilitates their apoptosis (Figure 4A) but prevents their autophagy, thereby allowing them to escape killing, as discussed in detail elsewhere [42,185–187]. The multidrug-resistant *K. pneumoniae* ST258 (KP35) inhibits Ca^{2+}-dependent calpains in macrophages and neutrophils to avoid ICK [188]. Thus, calpains specific to macrophages are critical players in generating an antimicrobial immune response to clear the infection. Microbes have developed different strategies to hijack this system for their growth and multiplication.

In addition to different TLRs, receptor activator of NF-κB ligand (RANKL) also induces calpain overactivity in macrophages, playing a crucial role in NF-κB activation and promoting a pro-inflammatory M1 macrophage phenotype that supports osteoclastogenesis or bone formation [189,190]. Moreover, macrophage-specific RANK-RANKL interactions in immunosuppressive TIME of different cancers may convert a cold tumor to a hot (immune-responsive) tumor via converting immunosuppressive M2 macrophages (that express RANK) to pro-inflammatory/antitumor M1 macrophages (Figure 4A) [191]. Macrophage-specific RANK-RANKL interaction/stimulation has also been observed to polarize M2 macrophages into M1 macrophages during *Leishmania major* infection, facilitating the clearance of the pathogen by generating ROS and NO· (Figure 4A) [192]. This further supports the notion that macrophage calpains are crucial in clearing infections by enhancing their antimicrobial and pro-inflammatory activities. The deletion of calpain 1 prevents the polarization of M0 macrophages to pro-inflammatory M1 macrophages, which are critical for generating a pro-inflammatory immune response (Figure 4A) [193]. Calpain activation in macrophages regulates NF-κB- and PI3K/AKT1-dependent pro-inflammatory immune responses, such as ROS, NO·, IL-1α, TNF-α, IL-12, and IL-23 generation, which are critical for clearing infections and inflammatory disease pathogenesis (Figure 4A).

The nicotinic acetylcholine receptor alpha 1 (nAChRα1) stimulation in renal macrophages also activates calpain 1 and 2 activity to induce their pro-inflammatory phenotype and function, which is observed in the experimental mouse model of chronic hypercholesterolemic nephropathy [194]. Interestingly, nAChRa1 is not highly expressed in other macrophages. However, α7nAchRs are highly expressed on macrophages and other innate immune cells, where their stimulation with Acetylcholine (Ach) and nicotine, two endogenous and exogenous ligands, suppresses the pro-inflammatory immune response in diverse inflammatory conditions, including pain and sepsis [195–197]. The α7nAchR activation-mediated anti-inflammatory effects involve calpain inhibition, as it decreases cytosolic Ca^{2+} level in alveolar macrophages activated by the extracellular adenosine triphosphate (ATP) [198]. The extracellular ATP acts as a danger signal and potent inflammatory mediator by acting on extracellular P2X and P2Y purine receptors, including P2X7, which is responsible for generating local and systemic inflammation that leads to organ damage, as seen during sepsis [199–201]. The ATP-P2X7 interaction on macrophages also induces calpain activation

due to cytosolic Ca^{2+} increase, inducing unconventional protein secretion, which is followed by their necrosis and NLRP3 inflammasome activation, inducing IL-1β secretion [202]. Moreover, this ATP-induced NLRP3 activation and IL-1β secretion involves a calpain-dependent vesicle-mediated secretion pathway. Moreover, transgenic mice overexpressing calpastatin (natural negative regulators of calpains) subjected to an anterior coronary artery ligation-induced myocardial infarction (MI) exhibit higher mortality at six weeks than wild-type (WT) mice with a high incidence of cardiac rupture during the first week post-MI [203]. This is due to decreased infiltration of CD4+T cells (CD8+T cell infiltration remained unchanged) and increased infiltration of monocyte/macrophages, which are defective in undergoing anti-inflammatory M2 macrophage proliferation and exerting their wound-healing action [203]. However, it is essential to note that calpastatin overexpression and calpain knockout may employ distinct mechanisms to exert their immunoregulatory effects under various inflammatory conditions.

Obesity has taken the shape of a pandemic and is also immunologically characterized as a condition of chronic low-grade systemic inflammation, affecting every target organ, including the immune system [204,205]. Macrophages are also considered critical players of obesity-associated immune dysregulation [206,207]. Calpains are involved in the lipid uptake process in macrophages due to the increased activity of the calcium-sensing receptor (CaSR) by extracellular calcium ions in the adipose tissue, leading to the generation of a pro-inflammatory adipose tissue environment [208,209]. Moreover, calpain 1 KO has protected high-fat-diet (HFD)- fed mice from developing liver inflammation, as indicated by decreased levels of oxidized low-density lipoprotein (oxLDL), malondialdehyde (MDA), TNF-α, and IL-6 [210]. Even in apolipoprotein E (ApoE) KO mice subjected to HFD-induced obesity, calpain 1 is critical for inflammatory liver damage [211]. Thus, local and systemic macrophages infiltrate the adipose tissue and other organs, such as the liver and kidneys, supporting a pro-inflammatory environment via calpain activation and the overactivation of calpain-dependent pro-inflammatory signaling events, which generate pro-inflammatory cytokines and molecules [212,213]. Moreover, obesity is a critical risk factor for inflammaging, and specific calpain inhibition has protected kidneys from inflammaging via various anti-inflammatory mechanisms, including the reduced production of pro-inflammatory molecules and cytokines from macrophages related to NF-κB and NLRP3 inflammasome activation [214]. Hence, calpain targeting in macrophages may exert anti-inflammatory action during acute and chronic inflammatory conditions depending on the disease type and organ-specificity of the macrophage. Therefore, further studies are needed in the field.

3.3.2. Neutrophils

are the most abundant (50-70%) leukocytes in human blood, the first innate immune cells to migrate to the site of infection and inflammation, where they counteract external or endogenous inflammatory threats. The constitutive calpain expression and activity in resting neutrophils negatively regulate their protrusion and migration, and specific calpain 1 inhibition induces neutrophil polarization and chemokinesis [215,216]. Furthermore, aging neutrophils in homeostatic or *in vitro* culture conditions undergo apoptosis due to calpain-dependent rearrangements in the protein composition and structure of the plasmalemmal cytoskeleton, such as the dissociation of proteins from F-actin and the loss of α-actin and ezrin proteins, two actin-binding, membrane-anchoring proteins (Figure 4B) [217]. This constitutive apoptosis of aging neutrophils occurs due to a synergism between cytosolic calpains and the proteasome, which is downstream of caspases and critical for limiting inflammation by supporting inflammation resolution as macrophages clear these apoptotic neutrophils via a process called efferocytosis [217,218]. Failure to resolve the inflammatory phase can induce a chronic stage of inflammation or acute tissue/organ damage, as seen in patients with sepsis [219–221].Thus, calpains are critical for neutrophil homeostasis, and defective calpain signaling may imbalance neutrophil-mediated immune homeostasis or immune regulation, including the death and clearance of senescent neutrophils and their clearance via efferocytosis.

On the other hand, specific knockout of calpain 1 and 2 in MIC, including neutrophils, decreased their infiltration and antimicrobial function at the site of acute bacterial peritonitis [43]. Thus, under homeostasis, calpains serve as negative regulators of neutrophil chemokinesis; however, during infection, inflammation, or in the presence of IL-8 and formyl-Met-Leu-Phe (fMLP), calpains are required for their chemotaxis and migration to the site [43,215]. Moreover, during infection, an increase in cytosolic Ca^{2+} of neutrophils activates calpains for their cell spreading (this process is critical for their transendothelial migration and chemotaxis), phagocytic activity, including phagocytic uptake and ROS-dependent intracellular killing of the phagocytosed pathogens (Figure 4B) [43,222,223]. Calpain activation weakens the nuclear envelope by degrading nesprin-1 (an outer nuclear membrane protein) [224]. Additionally, it induces nuclear condensation in response to increased cytosolic Ca^{2+}-mediated neutrophil extracellular trap (NET) formation or NETosis, triggered by peptidyl arginine deiminase-4 (PAD4)-mediated histone citrullination (Figure 4B) [224–226]. Furthermore, shear stress induces NETosis that further increases in response to NETosis inducing agents, such as ATP and lipopolysaccharide (LPS) by activating mechanosensitive ion channel Piezo1 [227]. Piezo1 activation induces calpain activity, which remodels cytoskeletal architecture leading to NETosis (Figure 4B) [227]. NETosis is a critical indicator of inflammation severity and also mediates resolution of inflammation, thereby maintaining the immune homeostasis [226,228]. Therefore, calpain inhibition during inflammatory conditions suppresses inflammatory tissue damage resulting due to exaggerated neutrophil infiltration and their inflammatory functions, such as ROS generation, NETosis, and other inflammatory molecules [229,230]. Hence, impact of calpains on neutrophils' functions and behavior is critical to study for better understanding inflammatory and immunoregulatory processes.

3.3.3. DCs

DCs are potent antigen-presenting cells (APCs) that regulate innate and adaptive immune responses under diverse immunological conditions, including tolerance, infections, and inflammatory diseases such as autoimmunity and cancer [162,231–233]. DCs also express the calpain system, which regulates their motility by cleaving actin filaments, the Wiskott-Aldrich Syndrome protein (WASP), β2 integrins, talin, paxillin, and vinculin in their podosomes, thereby controlling the composition and turnover of these structures [234]. Ca^{2+} signaling is critical for DC maturation; however, its effect on cytosolic calpains in this process remains to be explored [235].

Calpain inhibition in primary murine DCs inhibits their transendothelial migration, or diapedesis, to enter lymph nodes and activate antigen-specific T and B cells, which can delay the generation of a protective immune response against infections and vaccines (Figure 4C) [234,236–238]. Thus, calpains are critical for DC diapedesis (Figure 4C). Moreover, the presence of protein tyrosine phosphatase nor receptor type 22 (*PTPN22*)-encoded Lyp phosphatase (Lyp620W) variant increases the risk of autoimmunity by promoting the calpain-mediated cleavage of Lyp/Pep (Pep is the human PTP ortholog in mouse) (PTPN22), decreasing Lyp/Pep levels in hyperresponsive lymphocytes and DCs during steady state [239,240]. It is well established that PTPN22 single-nucleotide polymorphisms (SNPs) are associated with several autoimmune diseases, including rheumatoid arthritis (RA), type 1 diabetes mellitus (T1DM), and systemic lupus erythematosus (SLE) [241]. Furthermore, the Janus kinase 2/calpain pathway, in response to platelet-activating factor (PAF), activates PTPIB in monocyte-derived DCs (moDCs), which negatively regulates IL-6 production [242]. As PAF and PTP1B exert immunoregulatory action on different immune cells, the PAF-calpain-PTP1B axis must be explored to understand immune homeostasis and its dysregulation during inflammatory and infectious diseases [243–245]. The immunosuppressive plasmacytoid DCs (pDCs) in human lung cancer TME secrete mature IL-1α due to the activation of absent in melanoma-2 (AIM-2)-like receptors (ALRs)-based inflammasomes, which increase intracellular Ca^{2+} activating cytosolic calpains to generate mature IL-1α [246]. Thus, calpains regulate the transendothelial migration and immunoregulatory actions of DCs during inflammatory and autoimmune conditions, which warrants further exploration in response to different PRR and antigen stimuli.

3.3.4. Mast Cells

Mast cells are critical immunoregulatory innate immune cells, primarily known for their role in allergic diseases, such as allergic asthma and dermatitis, as well as anaphylaxis. However, with advances in immunology, their immunological territory has expanded to include infectious diseases, cancers, neurodegeneration, and autoimmunity [160,247–252]. The mast cell calpain system is a critical player in immunoglobulin E (IgE)-mediated allergic immune responses, as its inhibition blocks IgE-mediated mast cell degranulation and NF-κB-dependent production of pro-inflammatory cytokines, and attenuates IgE-mediated late-phase cutaneous anaphylaxis (Figure 4D) [253]. Activation of mast cells and their calpains in response to glia maturation factor (GMF), along with microglia activation in methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced mouse model of Parkinson's disease (PD), promotes dopaminergic neuron degeneration and progression of the disease (Figure 4D.) [254]. Calpain inhibition in the MPTP-induced mouse model of PD has been shown to protect against inflammatory dopaminergic neuronal loss and improve behavioral outcomes [255]. In addition, mast cells are also critical in TBI-induced neuroinflammation, and calpains have been shown to play a role in this process; therefore, it would be interesting to explore the immune cell-specific (including mast cell) roles of calpains in neuroinflammatory processes [256–259]. Moreover, calpains are also involved in mast cell adhesion and migration; therefore, their targeting may represent a novel therapeutic approach to control overactive mast cells in different inflammatory diseases [260].

4. Calpains in Innate Lymphoid Cells (ILCs)

Phenotypically, ILCs appear as lymphoid cells but do express specific antigen (Ag) receptors, such as T cell receptor (TCR) and B cell receptor (BCR) of T and B cells [261]. There are three major types of ILCs: (1) group 1 ILCs, which include natural Killer (NK) cells and ILC1s, secrete IFN-γ and exhibit cytotoxic action; (2) group 2 ILCs, which include ILC2s and secrete type 2 cytokines; and (3) group 3 ILCs, which include lymphoid tissue inducer (LTi) cells and ILC3s, which produce IL-22 and IL-17 [261]. The details of ILCs, their role in immunity, infectious, and inflammatory diseases, along with their interaction with other immune cells, such as adaptive immune cells, are discussed elsewhere [261–265]. Notably, only NK cells exhibit cytotoxic action; therefore, ILC1s, ILC2s, and ILC3s are considered helper-like ILCs [266]. Studies have indicated the presence of the calpain system in lymphoid cells; however, except for NK cells, their expression in other ILCs has not been studied yet [267,268]. The calpains of activated NK cells play a critical role in the apoptosis of their target cells [268]. On the other hand, calpain inhibition in activated human NK cells enhances their cytotoxic action by preventing calpain-dependent protein kinase C (PKC) proteolysis [269]. Interestingly, increased Ca^{2+} influx enhances NK-mediated cytotoxic action, which may regulate early granzyme (Gzm) and perforin, as well as later death receptor-mediated killing of cancer and virus-infected cells [270–272]. However, data are limited regarding calpain expression and function in determining the role of ILCs in inflammation and immunoregulation, which requires further exploration.

5. Calpains in T Cells

T cells, including helper, regulatory, and cytotoxic T cells, play a critical role in maintaining immune homeostasis, and their dysfunction is associated with several autoimmune diseases and cancers [273–276]. Calpain 1 null mice exhibit consistent expansion in their splenic white pulp (lymphoid hyperplasia) due to an increase in the number and size of follicles and periarteriolar lymphatic sheets (PALS) [277]. Lymphoid hyperplasia with marked plasmacytosis is also observed in the lymph nodes of calpain 1 null mice, and these mice exhibit multi-organ lymphoid infiltration. T cell and NK cell compartments are reduced in calpain 1 null mice (Figure 5A) [277]. The expression of the calpain system in naïve T cells, including several T cell lines, and its upregulation upon T cell stimulation, including anti-CD3 stimulation, has been reported [267,278]. Anti-CD3 monoclonal antibody-mediated activation of T cells activates the calpain system, which cleaves α-actinin, a critical

component for actin cytoskeletal assembly and pseudopod formation in activated T cells (Figure 5B) [279].
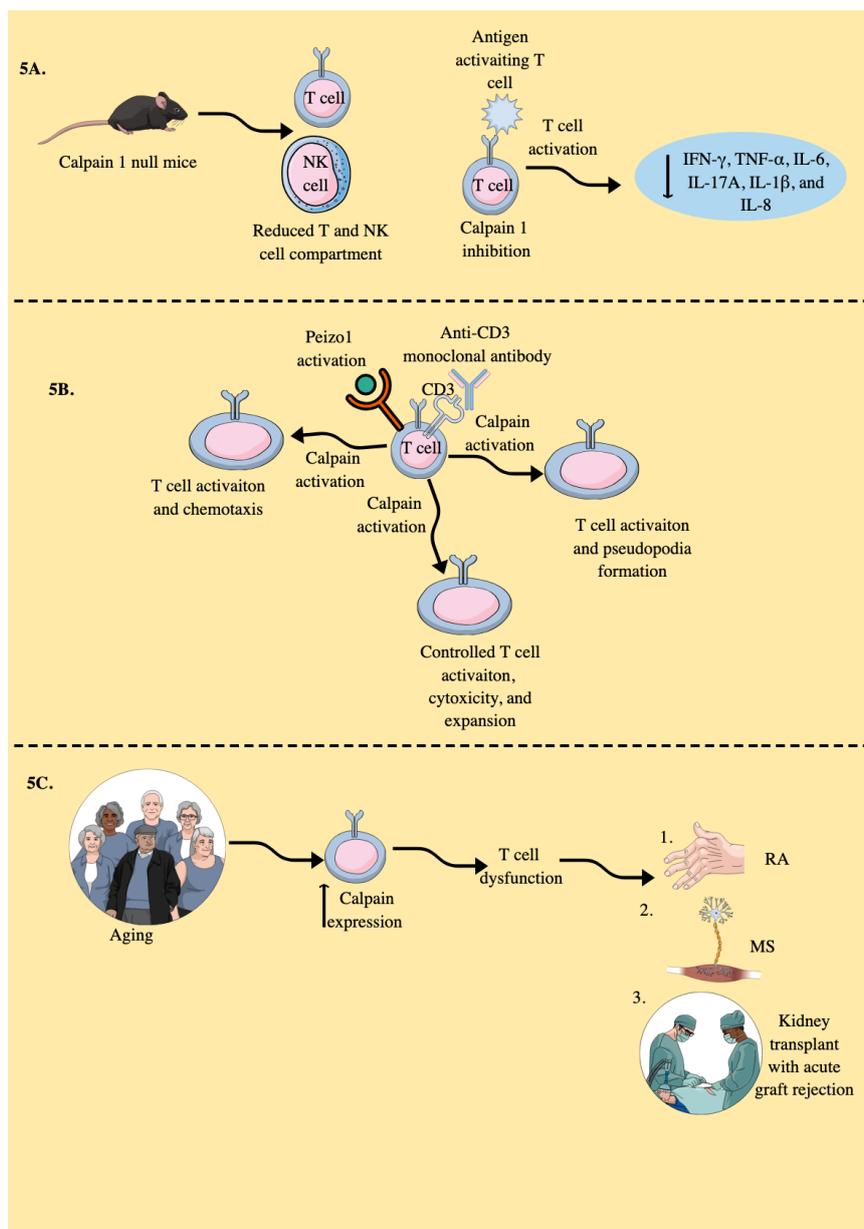

Figure 5. Calpains in T cells. **5A.** Calpain 1 null mice have reduced NK and T cell compartment. Moreover, T cells lacking calpain 1 upon activation secrete lower levels of IFNs and pro-inflammatory cytokines. **5B.** T cell activation response to anti-CD3 monoclonal antibody activates calpains, which is critical for their activation, pseudopodia formation, and mobility. Furthermore, calpain activation regulates their expansion and cytotoxicity. T cell peizo1 activation also regulates their chemotaxis and activation via calpain activity. **5C.** T cells of older people over express calpains, which may induce their dysfunction. For example, T cells isolated from patients with RA overexpress calpains and T cell infiltrated in acute graft rejection in patients undergoing kidney transplantation also overexpress calpains. Details are mentioned in the text.

Even resting CD4$^+$ and CD8$^+$T cells constitutively express the calpain system, and active calpastatin keeps a check on calpains to avoid their excessive proliferation and the release of various cytokines [280]. For example, calpain 1 inhibition results in the inhibition of various cytokines in stimulated peripheral blood monocytes (PBMCs), including IFN-α, TNF-α, IL-6, IL-17A, IL-1β, and IL-8 (Figure 5A) [280]. The stimulation of T cells with ionomycin or their adherence to fibronectin

activates calpains, which cleave PTP1B to generate active PTP1B, thereby serving as an intracellular checkpoint that limits their expansion and cytotoxicity [281,282]. The $\alpha4\beta1$ or $\alpha5\beta1$ integrin binds to fibronectin in appropriately stimulated T cells, also activating their calpains. The accumulation of calpain 2 and PTP1B at sites of focal contact formation has been reported [283]. The inhibition of calpain in T cells impairs their ability to adhere to and spread on immobilized fibronectin.

Moreover, activation of calpains in T cells upon antigen stimulation degrades TCR-associated zeta chain-associated protein kinase-70 (ZAP-70), which occurs in parallel with TCR internalization and degradation, indicating that calpain activation might control their overactivation, such as overexpansion and cytotoxicity (Figure 5B) [284]. For example, the negative regulation of ZAP-70 serves as a mechanistic basis for the differential expression of CD4$^+$ and CD8$^+$T cells in thymic and mature T cells [285]. Mechanosensor peizo1 also plays a critical role in T cell activation, which, upon activation, induces Ca^{2+} influx and calpain activation, thereby further organizing the cortical actin scaffolds required for optimal TCR activation (Figure 5B) [286]. Moreover, this peizo1 and calpain axis also regulates T cell chemotaxis (Figure 5B) by inducing an integrin called lymphocyte function-associated antigen 1 (LFA-1) at the leading edge of the chemotactic human T cells [287]. Thus, calpain-mediated talin cleavage is critical for LFA-1 activation. However, calpain 4, the regulatory component of calpains 1 and 2, is not essential for LFA-1-mediated adhesion, conjugation, or migration of CD4$^+$T cells under normal conditions [288].

The strong LFA-1-dependent adhesiveness of T$_{regs}$ to DCs is partly dependent on their lower calpain activities [289]. The T$_{regs}$' adhesion to DCs sequesters Fascin-1 (FSCN-1), an actin-bundling protein (critical for immunological synapse formation), and skews Fascin-1-dependent actin polarization in DCs toward the T$_{regs}$' adhesion zone [289]. This T$_{reg}$-DC interaction, or immune synapse, which is major histocompatibility complex class II (MHC-II) independent, induces lethargy in DCs, rendering them unable to induce potent T cell priming that supports the T$_{reg}$-mediated stage of immunosuppression or immunoregulation [289,290]. Talin1 is critical for TCR-induced adhesion of T cells to ICAM-1 and T cell-antigen-presenting cell (APC) conjugation or immune synapse formation [291]. However, cleavage of talin by calpains is critical for focal adhesion disassembly. It serves as a rate-limiting step during adhesion turnover, as this process also affects the disassembly of other adhesion components, including paxillin, vinculin, and zyxin [292]. For example, the talin-vinculin axis is a key mechanosensing component of cellular focal adhesions, as vinculin molecules bundle actin and localize to focal adhesions in a force-independent manner, requiring talin [293,294]. Thus, calpains are critical regulators of T cell activation, proliferation, and migration/chemotaxis in response to various routes of stimulation, which warrants further study due to the emergence of contradictory findings.

The expression of calpain in T cells increases with aging, indicating its role in T cell (CD4$^+$ and CD8$^+$) dysfunction, such as proliferation and associated pro-inflammatory functions (Figure 5C) [295]. For example, the cell cycle of CD4$^+$T cells in the healthy elderly population is significantly shorter than that of the young population, due to the overexpression of cyclin D, which is attributed to their lowered degradation by calpains [296]. Thus, it will be interesting to investigate calpain expression in T cells isolated from healthy elderly individuals and those with various diseases, such as cancer, neurodegenerative diseases, and metabolic syndrome, to explore their specific roles in regulating T cell-mediated immunity (TCMI) in the aging population. T cell stimulation also increases calpain secretion through their *ATP-binding cassette transporter* (ABCA1) transporters, which may modify the inflammatory immune environment [278,297]. For example, extracellular calpain activates anti-inflammatory TGF-β, inactivates pro-inflammatory chimerins, decreases IL-17 expression in murine Th17 cells by inducing the shedding of TLR2, helps in the regeneration of injured epithelium, and supports angiogenesis [297–303].

The calpain activity in T cells isolated from patients with rheumatoid arthritis (RA) is higher than that of healthy controls (Figure 5C) [304]. Moreover, RA patients develop calpastatin autoantibodies, and their incidence is higher than in other patients with systemic autoimmune diseases, such as systemic lupus erythematosus (SLE, 27%), polymyositis/dermatomyositis (24%),

systemic sclerosis (38%), and overlap syndrome (29%) [305,306]. The inhibition of calpains with a membrane-permeable cysteine protease inhibitor (E-64-d) in experimental arthritis models served as a therapeutic targeting. Thus, the calpain-calpastatin system may play a critical role in inflammatory cascades associated with autoimmune diseases. For example, the calpastatin-calpain balance is disturbed during Th1, Th2, and Th17 development, as calpastatin overexpression or calpain inhibition with E-64-d suppresses IL-6 and IL-17 production by Th cells and IL-6 production by fibroblasts, due to reduced RORγt expression and STAT3 phosphorylation [307]. Patients undergoing kidney transplants with acute graft rejection exhibit overexpression and activation of calpain-1 in their infiltrating T cells (Figure5C) [308]. Even calpain inhibition, achieved by silencing its small regulatory subunit (CPNS), inhibits Th17 development. Conversely, calpastatin overexpression inhibits IL-17 production from Th17 cells by over activating STAT-5 signaling. Thus, the calpain system must be studied during helper T (Th) cell polarization, as it is critical to immune responses during diverse inflammatory conditions, such as cancer, autoimmunity, and infectious diseases.

Calpain inhibition in mice with skin transplants increased the duration of skin allograft survival and diminished T cell infiltration into the allograft. It is interesting to note that calpastatin overexpression in T cells decreased the calpain level and their migration but increased their proliferation due to amplified IL-2 signaling via stabilization of IL-2R common γ-chain [308]. Thus, calpain inhibition delays allograft rejection by decreasing T cell infiltration but not their proliferation. Moreover, increased calpain activity is associated with increased levels of pro-inflammatory Th1 cells in patients with multiple sclerosis (MS) (Figure 5C), and calpain inhibitor treatment to PBMCs isolated from patients with MS decreases T cell proliferation, elevates their indoleamine 2,3-dioxygenase (IDO) levels, and downregulates Th1/Th17 inflammatory cytokines [309,310]. Moreover, IDO is a critical immunosuppressive molecule that catabolizes cytosolic tryptophan to induce immunosuppressive effects, including the induction of T_{regs} [311,312]. Thus, T cell-specific calpain inhibition can decrease pro- and autoinflammatory functions of T cells during MS. Hence, calpains govern the immunoregulatory and pro-inflammatory functions of T cells, including their polarization into different Th phenotypes, which must be further explored to understand their roles in various T cell-mediated inflammatory conditions, such as GVHD, autoimmunity, and cancers.

6. Calpains in B Cells

Ca^{2+} signaling in response to B cell receptor (BCR), PRR, cytokine, and chemokine signaling is critical for survival, proliferation, differentiation, and migration to lymphoid and target organs; therefore, its disruption may induce cell death or abnormal proliferation, as seen in different leukemias [313,314]. Calpains are Ca^{2+}-dependent cytosolic cysteine proteases that are also expressed in B cells, and their expression increases in CD19+B cells of aging individuals [295]. Calpain-1 null mice exhibit an overall increase in the number of B-cell lineage cells in the spleen and bone marrow, along with an increased myeloid-specific GR1+, CD11b+ granulocytic cell lineage and megakaryocytic CD41+ cells [277]. Notably, pancreatic islet B cells only express calpain 1 (μ-calpain) [315]. Calpain activation downstream of BCR signaling is critical for B cell clonal deletion, a process that eliminates autoreactive B cells, and the establishment of the B cell repertoire, which occurs due to the activation of caspase 7 (CASP7). In contrast, overexpression of its inhibitor (calpastatin) inhibits BCR-induced apoptosis in immunoglobulin M-positive (IgM+) cells [316,317]. Moreover, CD40 ligation during BCR signaling downregulates calpastatin levels, which support calpain activation in controlling B cell clonal deletion and establishing the B cell repertoire [317]. Activated calpains also cleave the Myc protein, which stimulates B cell differentiation and amplifies Ca^{2+} signaling, and its dysregulation may lead to various B cell malignancies [318–320]. Myc is also critical for the formation and maintenance of the germinal center (GC), and c-Myc+GC subpopulations of B cells may comprise a high risk for malignant transformation [321]. Hence, the calpain-Myc axis in GCs and B cell malignancies must be explored.

Overactive calpain-1 in chronic B-cell leukemia (B-CLL) and childhood acute leukemia blasts (ALL-B) has been reported to further increase with age and prevent their apoptosis [304,322].

However, there is hope in the form of calpain inhibitors. Treating these leukemia B cells with calpain inhibitors increases their apoptosis in a dose-dependent manner, offering a potential avenue for treatment. The increased calpain activity in B-CLL is associated with a decreased action of pro-apoptotic caspases, such as CASP3 and CASP9, and increased activity of anti-apoptotic Bcl-2 protein [323,324]. Moreover, calpain inhibition (calpain inhibitor II or CPI-2) also induced apoptosis among acute lymphoid leukemia (ALL) and non-Hodgkin's lymphoma B cells, which is dependent on caspase activation but not on the protein tyrosine kinases LYN or Bruton's tyrosine kinase (BTK) [325].

Furthermore, calpain-1 overexpression well correlates with phosphorylated zeta-associated protein 70 (pZAP-70) in B-CLL lymphocytes. Interestingly, the proportion of CD19+ B cells with overexpressed and active calpain-1 and pZap-70 significantly decreases in patients with B-CLL after successful therapy [323]. TLR2 activation in B cells during *S. flexneri* infection induces their apoptosis. Therefore, it is crucial to investigate calpain activation, as TLR2 activation induces calpain activation in different innate immune cells, as discussed earlier [326,327]. Understanding calpain activity downstream of BCR and PRR signaling in B cells under diverse infectious and inflammatory conditions, such as cancers and autoimmunity, is of utmost importance. This knowledge can help us develop more effective vaccine and immunotherapy candidates to target infectious diseases and cancers, underscoring the urgency and significance of our research.

7. Future Perspectives and Conclusion

The calpain system is present in all innate adaptive immune cells and regulates their death, function, and migration/mobility to or away from the site of infection/inflammation. Moreover, their controlled activity is crucial for immune cell migration across both epithelial and endothelial barriers, suggesting that their epithelial and endothelial cell-specific targeting has the potential to treat inflammatory diseases. For example, different calpain-specific therapeutic strategies have been developed with better bioavailability and specificity of calpain inhibitors to target various inflammatory diseases, such as cancers, calpainopathies, neurodegenerative diseases, TBI, and several other inflammatory diseases [328–334]. Although calpain targeting is emerging as a novel pharmacotherapy for several inflammatory diseases, including cancers, data regarding immune cell-specific targeting are scarce. Therefore, immune cell-specific calpain system targeting may be a helpful approach to target dysregulated immune responses in an immune cell-specific manner. For example, DC-specific calpain activation during infectious diseases and cancers, where DC migration to LNs helps generate an antigen-specific immune response, such as effector T and B cells, will provide us with the opportunity to develop potent adjuvants to increase the efficacy of currently available vaccines and therapies. Even antimicrobial peptides (AMPs), such as cathelicidin LL-37, induce apoptosis in their target cells, including cancer cells and infected cells, by activating calpain along with the apoptosis-inducing factor (AIF) pathway [335,336]. Thus, AMP-mediated calpain upregulation can be utilized to combat intracellular infections and cancers in an immune cell-specific manner [337,338].

The selective deletion of calpain 1 and 2 in endothelial cells reduces cardiac fibrosis and hypertrophy, while also alleviating myocardial dysfunction by supporting angiogenesis and preventing apoptosis in mice with diabetes, a metabolic syndrome condition characterized by elevated chronic systemic inflammation [339]. Moreover, calpain inhibition in pancreatic islet cells increases insulin release by enhancing exocytosis of insulin granules [340]. Thus, calpain inhibition may be protective in metabolic syndrome associated with diabetes; therefore, it would be interesting to see how it affects individual immune cells responsible for systemic chronic inflammation seen in these patients. Cytosolic Ca^{2+} influx is a critical determinant of calpain activity, and studies have shown that glucagon-like peptide-1 receptor (GLP-1R) agonists, which are commonly prescribed for weight loss and anti-T2DM treatment, are associated with a reduced risk of hypocalcemia but an increased risk of hypercalcemia [341]. Moreover, GLP-1 Agonists have been shown to exert anti-inflammatory and immune-modifying effects through the neuroimmune axis and a direct effect on

different immune cells [342]. Interestingly, cytosolic Ca^{2+} influx also supports the GLP-1 release from enteroendocrine or L cells [343]. Therefore, it would be interesting to investigate any GLP-1/GLP-1R/calpain axis in immune cells due to its impact on cytosolic Ca^{2+}, which determines survival, proliferation, and immunological functions immune cells, as well as its release.

In conclusion, the immune cell-specific calpain system (calpain 1 and 2) is an area that is starting to gain recognition. However, further exploration is crucial to fully understand and develop immune cell-specific targeting of this system to control inflammatory diseases and associated dysregulation. The functional regulation of every immune cell, like that of any other cell, is governed and affected by the cytosolic Ca^{2+} flux, a critical regulator of calpain. Therefore, calpains must be studied in the context of specific immune cell function and regulation. They not only cleave cytoskeletal proteins but are also associated with NLRP3 inflammasome activation, IL-1β, adhesion molecule expression, antigen presentation, and the cleavage of several other non-cytoskeletal proteins. Hence, a calpain lens is crucial for understanding inflammation and immune dysregulation in order to reestablish immune homeostasis. The potential for further exploration and the development of immune cell-specific targeting strategies make this an exciting area for future research.

References

1. Kumar, V.; Stewart, J. H. t., Immune Homeostasis: A Novel Example of Teamwork. *Methods Mol Biol* **2024,** 2782, 1-24.

2. Nobs, S. P.; Kopf, M., Tissue-resident macrophages: guardians of organ homeostasis. *Trends in Immunology* **2021,** 42, (6), 495-507.

3. Gray, J. I.; Farber, D. L., Tissue-Resident Immune Cells in Humans. *Annu Rev Immunol* **2022,** 40, 195-220.

4. Gebhardt, T.; Palendira, U.; Tscharke, D. C.; Bedoui, S., Tissue-resident memory T cells in tissue homeostasis, persistent infection, and cancer surveillance. *Immunol Rev* **2018,** 283, (1), 54-76.

5. Parijs, L. V.; Abbas, A. K., Homeostasis and Self-Tolerance in the Immune System: Turning Lymphocytes off. *Science* **1998,** 280, (5361), 243-248.

6. Chatenoud, L., Teaching the immune system "self" respect and tolerance. *Science* **2014,** 344, (6190), 1343-1344.

7. Halper-Stromberg, A.; Jabri, B., Maladaptive consequences of inflammatory events shape individual immune identity. *Nature Immunology* **2022,** 23, (12), 1675-1686.

8. Goll, D. E.; Thompson, V. F.; Li, H.; Wei, W.; Cong, J., The calpain system. *Physiol Rev* **2003,** 83, (3), 731-801.

9. Hanna, R. A.; Campbell, R. L.; Davies, P. L., Calcium-bound structure of calpain and its mechanism of inhibition by calpastatin. *Nature* **2008,** 456, (7220), 409-12.

10. Wendt, A.; Thompson, V. F.; Goll, D. E., Interaction of calpastatin with calpain: a review. *Biol Chem* **2004,** 385, (6), 465-72.

11. Glading, A.; Lauffenburger, D. A.; Wells, A., Cutting to the chase: calpain proteases in cell motility. *Trends in Cell Biology* **2002,** 12, (1), 46-54.

12. Franco, S. J.; Huttenlocher, A., Regulating cell migration: calpains make the cut. *Journal of Cell Science* **2005,** 118, (17), 3829-3838.

13. Abe, K.; Takeichi, M., NMDA-Receptor Activation Induces Calpain-Mediated β-Catenin Cleavages for Triggering Gene Expression. *Neuron* **2007,** 53, (3), 387-397.

14. Bollino, D.; Balan, I.; Aurelian, L., Valproic acid induces neuronal cell death through a novel calpain-dependent necroptosis pathway. *J Neurochem* **2015,** 133, (2), 174-86.

15. Davis, M. A.; Fairgrieve, M. R.; Den Hartigh, A.; Yakovenko, O.; Duvvuri, B.; Lood, C.; Thomas, W. E.; Fink, S. L.; Gale, M., Calpain drives pyroptotic vimentin cleavage, intermediate filament loss, and cell rupture that mediates immunostimulation. *Proceedings of the National Academy of Sciences* **2019,** 116, (11), 5061-5070.

16. Kumar, V., Toll-like receptors in immunity and inflammatory diseases: Past, present, and future. *Int Immunopharmacol* **2018,** 59, 391-412.

17. Kumar, V., Inflammation research sails through the sea of immunology to reach immunometabolism. *International Immunopharmacology* **2019,** 73, 128-145.

18. Kumar, V., Stewart IV, JH, Toll-Like Receptors in Immunity and Inflammation. In *Thirty Years since the Discovery of Toll-Like Receptors*, Kumar, V., Ed. IntechOpen: Rijeka, 2024.

19. Kumar, V.; Stewart, J. H. t., cGLRs Join Their Cousins of Pattern Recognition Receptor Family to Regulate Immune Homeostasis. *Int J Mol Sci* **2024,** 25, (3).

20. Gong, T.; Liu, L.; Jiang, W.; Zhou, R., DAMP-sensing receptors in sterile inflammation and inflammatory diseases. *Nat Rev Immunol* **2020,** 20, (2), 95-112.

21. Kumar, V., Toll-Like Receptors in Adaptive Immunity. *Handb Exp Pharmacol* **2022,** 276, 95-131.

22. Chou, W. C.; Jha, S.; Linhoff, M. W.; Ting, J. P., The NLR gene family: from discovery to present day. *Nat Rev Immunol* **2023,** 23, (10), 635-654.

23. Brown, G. D.; Willment, J. A.; Whitehead, L., C-type lectins in immunity and homeostasis. *Nat Rev Immunol* **2018,** 18, (6), 374-389.

24. Canton, J.; Neculai, D.; Grinstein, S., Scavenger receptors in homeostasis and immunity. *Nat Rev Immunol* **2013,** 13, (9), 621-34.

25. Kumar, V., The Trinity of cGAS, TLR9, and ALRs Guardians of the Cellular Galaxy Against Host-Derived Self-DNA. *Front Immunol* **2020,** 11, 624597.

26. Kumar, V., The complement system, toll-like receptors and inflammasomes in host defense: three musketeers' one target. *Int Rev Immunol* **2019,** 38, (4), 131-156.

27. Chen, R.; Zou, J.; Chen, J.; Zhong, X.; Kang, R.; Tang, D., Pattern recognition receptors: function, regulation and therapeutic potential. *Signal Transduction and Targeted Therapy* **2025,** 10, (1), 216.

28. Li, Y.; Slavik, K. M.; Toyoda, H. C.; Morehouse, B. R.; de Oliveira Mann, C. C.; Elek, A.; Levy, S.; Wang, Z.; Mears, K. S.; Liu, J.; Kashin, D.; Guo, X.; Mass, T.; Sebé-Pedrós, A.; Schwede, F.; Kranzusch, P. J., cGLRs are a diverse family of pattern recognition receptors in innate immunity. *Cell* **2023,** 186, (15), 3261-3276.e20.

29. Carroll, S. L.; Pasare, C.; Barton, G. M., Control of adaptive immunity by pattern recognition receptors. *Immunity* **2024,** 57, (4), 632-648.

30. Duxbury, Z.; Wu, C. H.; Ding, P., A Comparative Overview of the Intracellular Guardians of Plants and Animals: NLRs in Innate Immunity and Beyond. *Annu Rev Plant Biol* **2021,** 72, 155-184.

31. Kumar, V.; Stewart Iv, J. H., Pattern-Recognition Receptors and Immunometabolic Reprogramming: What We Know and What to Explore. *J Innate Immun* **2024,** 16, (1), 295-323.

32. Kumar, V., A STING to inflammation and autoimmunity. *J Leukoc Biol* **2019,** 106, (1), 171-185.

33. Li, D.; Wu, M., Pattern recognition receptors in health and diseases. *Signal Transduction and Targeted Therapy* **2021,** 6, (1), 291.

34. Okin, D.; Medzhitov, R., Evolution of inflammatory diseases. *Curr Biol* **2012,** 22, (17), R733-40.

35. Cooke, J. P., Inflammation and Its Role in Regeneration and Repair. *Circ Res* **2019,** 124, (8), 1166-1168.

36. Aurora, Arin B.; Olson, Eric N., Immune Modulation of Stem Cells and Regeneration. *Cell Stem Cell* **2014,** 15, (1), 14-25.

37. Kumar, V., Inflammasomes: Pandora's box for sepsis. *J Inflamm Res* **2018,** 11, 477-502.

38. Furman, D.; Campisi, J.; Verdin, E.; Carrera-Bastos, P.; Targ, S.; Franceschi, C.; Ferrucci, L.; Gilroy, D. W.; Fasano, A.; Miller, G. W.; Miller, A. H.; Mantovani, A.; Weyand, C. M.; Barzilai, N.; Goronzy, J. J.; Rando, T. A.; Effros, R. B.; Lucia, A.; Kleinstreuer, N.; Slavich, G. M., Chronic inflammation in the etiology of disease across the life span. *Nature Medicine* **2019,** 25, (12), 1822-1832.

39. Muszka, Z.; Jenei, V.; Mácsik, R.; Mezhonova, E.; Diyab, S.; Csősz, R.; Bácsi, A.; Mázló, A.; Koncz, G., Life-threatening risk factors contribute to the development of diseases with the highest mortality through the induction of regulated necrotic cell death. *Cell Death & Disease* **2025,** 16, (1), 273.

40. Wu, D.; Jin, Y.; Xing, Y.; Abate, M. D.; Abbasian, M.; Abbasi-Kangevari, M.; Abbasi-Kangevari, Z.; Abd-Allah, F.; Abdelmasseh, M.; Abdollahifar, M.-A.; Abdulah, D. M.; et al. Global, regional, and national incidence of six major immune-mediated inflammatory diseases: findings from the global burden of disease study 2019. *eClinicalMedicine* **2023,** 64.

41. Kong, F.; You, H.; Zheng, K.; Tang, R.; Zheng, C., The crosstalk between pattern-recognition receptor signaling and calcium signaling. *Int J Biol Macromol* **2021,** 192, 745-756.

42. Kumar, V.; Ahmad, A., Targeting calpains: A novel immunomodulatory approach for microbial infections. *Eur J Pharmacol* **2017,** 814, 28-44.

43. Kumar, V.; Everingham, S.; Hall, C.; Greer, P. A.; Craig, A. W. B., Calpains promote neutrophil recruitment and bacterial clearance in an acute bacterial peritonitis model. *European Journal of Immunology* **2014,** 44, (3), 831-841.

44. Chun, J.; Prince, A., TLR2-induced calpain cleavage of epithelial junctional proteins facilitates leukocyte transmigration. *Cell Host Microbe* **2009,** 5, (1), 47-58.

45. Deng, H.; Tian, X.; Sun, H.; Liu, H.; Lu, M.; Wang, H., Calpain-1 mediates vascular remodelling and fibrosis via HIF-1α in hypoxia-induced pulmonary hypertension. *J Cell Mol Med* **2022,** 26, (10), 2819-2830.

46. Lu, T.; Xu, Y.; Mericle, M. T.; Mellgren, R. L., Participation of the conventional calpains in apoptosis. *Biochim Biophys Acta* **2002,** 1590, (1-3), 16-26.

47. Horbach, N.; Kalinka, M.; Ćwilichowska-Puślecka, N.; Al Mamun, A.; Mikołajczyk-Martinez, A.; Turk, B.; Snipas, S. J.; Kasperkiewicz, P.; Groborz, K. M.; Poręba, M., Visualization of calpain-1 activation during cell death and its role in GSDMD cleavage using chemical probes. *Cell Chem Biol* **2025,** 32, (4), 603-619.e7.

48. Chai, R.; Li, Y.; Shui, L.; Ni, L.; Zhang, A., The role of pyroptosis in inflammatory diseases. *Frontiers in Cell and Developmental Biology* **2023,** Volume 11 - 2023.

49. Hou, J.; Hsu, J.-M.; Hung, M.-C., Molecular mechanisms and functions of pyroptosis in inflammation and antitumor immunity. *Molecular Cell* **2021,** 81, (22), 4579-4590.

50. Broz, P., Pyroptosis: molecular mechanisms and roles in disease. *Cell Research* **2025,** 35, (5), 334-344.

51. Mulla, J.; Katti, R.; Scott, M. J., The Role of Gasdermin-D-Mediated Pyroptosis in Organ Injury and Its Therapeutic Implications. *Organogenesis* **2023,** 19, (1), 2177484.

52. Schleimer, R. P.; Kato, A.; Kern, R.; Kuperman, D.; Avila, P. C., Epithelium: at the interface of innate and adaptive immune responses. *J Allergy Clin Immunol* **2007,** 120, (6), 1279-84.

53. Ioannidis, I.; Ye, F.; McNally, B.; Willette, M.; Flaño, E., Toll-Like Receptor Expression and Induction of Type I and Type III Interferons in Primary Airway Epithelial Cells. *Journal of Virology* **2013,** 87, (6), 3261-3270.

54. Yang, J.; Yan, H., Mucosal epithelial cells: the initial sentinels and responders controlling and regulating immune responses to viral infections. *Cellular & Molecular Immunology* **2021,** 18, (7), 1628-1630.

55. Bals, R.; Hiemstra, P. S., Innate immunity in the lung: how epithelial cells fight against respiratory pathogens. *European Respiratory Journal* 23, (2), 327-333.

56. Pott, J.; Hornef, M., Innate immune signalling at the intestinal epithelium in homeostasis and disease. *EMBO reports* **2012,** 13, (8), 684-698.

57. Hewitt, R. J.; Lloyd, C. M., Regulation of immune responses by the airway epithelial cell landscape. *Nature Reviews Immunology* **2021,** 21, (6), 347-362.

58. de Vries, M. H.; Kuijk, E. W.; Nieuwenhuis, E. E. S., Innate immunity of the gut epithelium: Blowing in the WNT? *Mucosal Immunology* **2025**.

59. Constant, D. A.; Nice, T. J.; Rauch, I., Innate immune sensing by epithelial barriers. *Curr Opin Immunol* **2021,** 73, 1-8.

60. Johnston, S. L.; Goldblatt, D. L.; Evans, S. E.; Tuvim, M. J.; Dickey, B. F., Airway Epithelial Innate Immunity. *Frontiers in Physiology* **2021,** Volume 12 - 2021.

61. Kato, A.; Schleimer, R. P., Beyond inflammation: airway epithelial cells are at the interface of innate and adaptive immunity. *Curr Opin Immunol* **2007,** 19, (6), 711-20.

62. Hui, C. C.; Yu, A.; Heroux, D.; Akhabir, L.; Sandford, A. J.; Neighbour, H.; Denburg, J. A., Thymic stromal lymphopoietin (TSLP) secretion from human nasal epithelium is a function of TSLP genotype. *Mucosal Immunol* **2015,** 8, (5), 993-9.

63. Ebina-Shibuya, R.; Leonard, W. J., Role of thymic stromal lymphopoietin in allergy and beyond. *Nature Reviews Immunology* **2023,** 23, (1), 24-37.

64. Hatton, C. F.; Botting, R. A.; Dueñas, M. E.; Haq, I. J.; Verdon, B.; Thompson, B. J.; Spegarova, J. S.; Gothe, F.; Stephenson, E.; Gardner, A. I.; et al. Delayed induction of type I and III interferons mediates nasal epithelial cell permissiveness to SARS-CoV-2. *Nature Communications* **2021,** 12, (1), 7092.

65. Sharma, M.; Sharma, S.; Roy, S.; Varma, S.; Bose, M., Pulmonary epithelial cells are a source of interferon-gamma in response to Mycobacterium tuberculosis infection. *Immunol Cell Biol* **2007,** 85, (3), 229-37.

66. Kulkarni, H. S.; Elvington, M. L.; Perng, Y.-C.; Liszewski, M. K.; Byers, D. E.; Farkouh, C.; Yusen, R. D.; Lenschow, D. J.; Brody, S. L.; Atkinson, J. P., Intracellular C3 Protects Human Airway Epithelial Cells from Stress-associated Cell Death. *American Journal of Respiratory Cell and Molecular Biology* **2019,** 60, (2), 144-157.

67. Kulkarni, D. H.; Starick, M.; Aponte Alburquerque, R.; Kulkarni, H. S., Local complement activation and modulation in mucosal immunity. *Mucosal Immunology* **2024,** 17, (4), 739-751.

68. Chaudhary, N.; Jayaraman, A.; Reinhardt, C.; Campbell, J. D.; Bosmann, M., A single-cell lung atlas of complement genes identifies the mesothelium and epithelium as prominent sources of extrahepatic complement proteins. *Mucosal Immunology* **2022,** 15, (5), 927-939.

69. Kulkarni, H. S.; Liszewski, M. K.; Brody, S. L.; Atkinson, J. P., The complement system in the airway epithelium: An overlooked host defense mechanism and therapeutic target? *J Allergy Clin Immunol* **2018,** 141, (5), 1582-1586.e1.

70. Sahu, S. K.; Ozantürk, A. N.; Kulkarni, D. H.; Ma, L.; Barve, R. A.; Dannull, L.; Lu, A.; Starick, M.; McPhatter, J. N.; Garnica, L.; Sanfillipo-Burchman, M.; Kunen, J.; Wu, X.; Gelman, A. E.; Brody, S. L.; Atkinson, J. P.; Kulkarni, H. S., Lung epithelial cell–derived C3 protects against pneumonia-induced lung injury. *Science Immunology* **2023,** 8, (80), eabp9547.

71. Bush, K. T.; Tsukamoto, T.; Nigam, S. K., Selective degradation of E-cadherin and dissolution of E-cadherin-catenin complexes in epithelial ischemia. *Am J Physiol Renal Physiol* **2000,** 278, (5), F847-52.

72. Nakajima, T.; Shearer, T. R.; Azuma, M., Loss of Calpastatin Leads to Activation of Calpain in Human Lens Epithelial Cells. *Investigative Ophthalmology & Visual Science* **2014,** 55, (8), 5278-5283.

73. Rasl, J.; Caslavsky, J.; Grusanovic, J.; Chvalova, V.; Kosla, J.; Adamec, J.; Grousl, T.; Klimova, Z.; Vomastek, T., Depletion of calpain2 accelerates epithelial barrier establishment and reduces growth factor-induced cell scattering. *Cellular Signalling* **2024,** 121, 111295.

74. Ahn, Y. J.; Kim, M. S.; Chung, S. K., Calpain and Caspase-12 Expression in Lens Epithelial Cells of Diabetic Cataracts. *American Journal of Ophthalmology* **2016,** 167, 31-37.

75. Biswas, S.; Harris, F.; Dennison, S.; Singh, J.; Phoenix, D. A., Calpains: targets of cataract prevention? *Trends in Molecular Medicine* **2004,** 10, (2), 78-84.

76. Gupta, P. D.; Johar, K.; Vasavada, A., Causative and preventive action of calcium in cataractogenesis. *Acta Pharmacologica Sinica* **2004,** 25, 1250-1256.

77. Biswas, S.; Harris, F.; Singh, J.; Phoenix, D., Role of calpains in diabetes mellitus-induced cataractogenesis: a mini review. *Molecular and cellular biochemistry* **2004,** 261, (1), 151-159.

78. Chen, L.; Yao, K.; Fu, Q. L., Potential immune involvement in cataract: from mechanisms to future scope of therapies. *Int J Ophthalmol* **2025,** 18, (3), 541-548.

79. Kumar, V., Toll-like receptors in the pathogenesis of neuroinflammation. *J Neuroimmunol* **2019,** 332, 16-30.

80. Kumar, V., Toll-like receptors in sepsis-associated cytokine storm and their endogenous negative regulators as future immunomodulatory targets. *International Immunopharmacology* **2020,** 89, 107087.

81. Burgueño, J. F.; Abreu, M. T., Epithelial Toll-like receptors and their role in gut homeostasis and disease. *Nature Reviews Gastroenterology & Hepatology* **2020,** 17, (5), 263-278.

82. Gribar, S. C.; Richardson, W. M.; Sodhi, C. P.; Hackam, D. J., No Longer an Innocent Bystander: Epithelial Toll-Like Receptor Signaling in the Development of Mucosal Inflammation. *Molecular Medicine* **2008,** 14, (9), 645-659.

83. Rakoff-Nahoum, S.; Paglino, J.; Eslami-Varzaneh, F.; Edberg, S.; Medzhitov, R., Recognition of Commensal Microflora by Toll-Like Receptors Is Required for Intestinal Homeostasis. *Cell* **2004,** 118, (2), 229-241.

84. Soranno, D. E.; Coopersmith, C. M.; Brinkworth, J. F.; Factora, F. N. F.; Muntean, J. H.; Mythen, M. G.; Raphael, J.; Shaw, A. D.; Vachharajani, V.; Messer, J. S., A review of gut failure as a cause and consequence of critical illness. *Critical Care* **2025,** 29, (1), 91.

85. Sha, Q.; Truong-Tran, A. Q.; Plitt, J. R.; Beck, L. A.; Schleimer, R. P., Activation of airway epithelial cells by toll-like receptor agonists. *Am J Respir Cell Mol Biol* **2004,** 31, (3), 358-64.

86. Tengroth, L.; Millrud, C. R.; Kvarnhammar, A. M.; Kumlien Georén, S.; Latif, L.; Cardell, L.-O., Functional Effects of Toll-Like Receptor (TLR)3, 7, 9, RIG-I and MDA-5 Stimulation in Nasal Epithelial Cells. *PLOS ONE* **2014,** 9, (6), e98239.

87. Invernizzi, R.; Lloyd, C. M.; Molyneaux, P. L., Respiratory microbiome and epithelial interactions shape immunity in the lungs. *Immunology* **2020,** 160, (2), 171-182.

88. Li, R.; Li, J.; Zhou, X., Lung microbiome: new insights into the pathogenesis of respiratory diseases. *Signal Transduction and Targeted Therapy* **2024,** 9, (1), 19.

89. Eckert, R. L.; Rorke, E. A., Molecular biology of keratinocyte differentiation. *Environ Health Perspect* **1989,** 80, 109-16.

90. Kumar, V., Going, Toll-like receptors in skin inflammation and inflammatory diseases. *Excli j* **2021,** 20, 52-79.

91. Cha, J.; Kim, T.-G.; Ryu, J.-H., Conversation between skin microbiota and the host: from early life to adulthood. *Experimental & Molecular Medicine* **2025,** 57, (4), 703-713.

92. Flowers, L.; Grice, E. A., The Skin Microbiota: Balancing Risk and Reward. *Cell Host & Microbe* **2020,** 28, (2), 190-200.

93. Tang, S.; Chen, T.; Yang, M.; Wang, L.; Yu, Z.; Xie, B.; Qian, C.; Xu, S.; Li, N.; Cao, X.; Wang, J., Extracellular calcium elicits feedforward regulation of the Toll-like receptor-triggered innate immune response. *Cell Mol Immunol* **2017,** 14, (2), 180-191.

94. Birla, H.; Xia, J.; Gao, X.; Zhao, H.; Wang, F.; Patel, S.; Amponsah, A.; Bekker, A.; Tao, Y.-X.; Hu, H., Toll-like receptor 4 activation enhances Orai1-mediated calcium signal promoting cytokine production in spinal astrocytes. *Cell Calcium* **2022,** 105, 102619.

95. Park, K. S.; Kim, S. H.; Das, A.; Yang, S.-N.; Jung, K. H.; Kim, M. K.; Berggren, P.-O.; Lee, Y.; Chai, J. C.; Kim, H. J.; Chai, Y. G., TLR3-/4-Priming Differentially Promotes Ca2+ Signaling and Cytokine Expression and Ca2+-Dependently Augments Cytokine Release in hMSCs. *Scientific Reports* **2016,** 6, (1), 23103.

96. Fitzgerald, K. A.; Kagan, J. C., Toll-like Receptors and the Control of Immunity. *Cell* **2020,** 180, (6), 1044-1066.

97. Tan, Y.; Zanoni, I.; Cullen, T. W.; Goodman, A. L.; Kagan, J. C., Mechanisms of Toll-like Receptor 4 Endocytosis Reveal a Common Immune-Evasion Strategy Used by Pathogenic and Commensal Bacteria. *Immunity* **2015,** 43, (5), 909-22.

98. Kagan, J. C.; Su, T.; Horng, T.; Chow, A.; Akira, S.; Medzhitov, R., TRAM couples endocytosis of Toll-like receptor 4 to the induction of interferon-beta. *Nat Immunol* **2008,** 9, (4), 361-8.

99. Schultz, T. E.; Mathmann, C. D.; Domínguez Cadena, L. C.; Muusse, T. W.; Kim, H.; Wells, J. W.; Ulett, G. C.; Hamerman, J. A.; Brooks, A. J.; Kobe, B.; Sweet, M. J.; Stacey, K. J.; Blumenthal, A., TLR4 endocytosis and endosomal TLR4 signaling are distinct and independent outcomes of TLR4 activation. *EMBO reports* **2025,** 26, (10), 2740-2766.

100. Stack, J.; Doyle, S. L.; Connolly, D. J.; Reinert, L. S.; O'Keeffe, K. M.; McLoughlin, R. M.; Paludan, S. R.; Bowie, A. G., TRAM is required for TLR2 endosomal signaling to type I IFN induction. *J Immunol* **2014,** 193, (12), 6090-102.

101. De Dios, R.; Nguyen, L.; Ghosh, S.; McKenna, S.; Wright, C. J., CpG-ODN-mediated TLR9 innate immune signalling and calcium dyshomeostasis converge on the NFκB inhibitory protein IκBβ to drive IL1α and IL1β expression. *Immunology* **2020,** 160, (1), 64-77.

102. O'Connor, P. M.; Lapointe, T. K.; Jackson, S.; Beck, P. L.; Jones, N. L.; Buret, A. G., Helicobacter pylori Activates Calpain via Toll-Like Receptor 2 To Disrupt Adherens Junctions in Human Gastric Epithelial Cells. *Infection and Immunity* **2011,** 79, (10), 3887-3894.

103. Bergounioux, J.; Elisee, R.; Prunier, A.-L.; Donnadieu, F.; Sperandio, B.; Sansonetti, P.; Arbibe, L., Calpain Activation by the Shigella flexneri Effector VirA Regulates Key Steps in the Formation and Life of the Bacterium's Epithelial Niche. *Cell Host & Microbe* **2012,** 11, (3), 240-252.

104. Lapaquette, P.; Fritah, S.; Lhocine, N.; Andrieux, A.; Nigro, G.; Mounier, J.; Sansonetti, P.; Dejean, A., Shigella entry unveils a calcium/calpain-dependent mechanism for inhibiting sumoylation. *Elife* **2017,** 6.

105. Fritah, S.; Lhocine, N.; Golebiowski, F.; Mounier, J.; Andrieux, A.; Jouvion, G.; Hay, R. T.; Sansonetti, P.; Dejean, A., Sumoylation controls host anti-bacterial response to the gut invasive pathogen Shigella flexneri. *EMBO Rep* **2014,** 15, (9), 965-72.

106. Karhausen, J.; Ulloa, L.; Yang, W., SUMOylation Connects Cell Stress Responses and Inflammatory Control: Lessons From the Gut as a Model Organ. *Front Immunol* **2021,** 12, 646633.

107. Eislmayr, K. D.; Nichols, C. A.; Liu, F. L.; Yuvaraj, S.; Babirye, J. P.; Roncaioli, J. L.; Vickery, J.; Barton, G. M.; Lesser, C. F.; Vance, R. E., Macrophages orchestrate elimination of Shigella from the intestinal epithelial cell niche via TLR-induced IL-12 and IFN-&#x3b3. *Cell Host & Microbe* **2025,** 33, (9), 1535-1549.e7.

108. Pore, D.; Mahata, N.; Pal, A.; Chakrabarti, M. K., 34 kDa MOMP of Shigella flexneri promotes TLR2 mediated macrophage activation with the engagement of NF-kappaB and p38 MAP kinase signaling. *Mol Immunol* **2010,** 47, (9), 1739-46.

109. Schuhmann, D.; Godoy, P.; Weiss, C.; Gerloff, A.; Singer, M. V.; Dooley, S.; Böcker, U., Interfering with interferon-γ signalling in intestinal epithelial cells: selective inhibition of apoptosis-maintained secretion of anti-inflammatory interleukin-18 binding protein. *Clin Exp Immunol* **2011,** 163, (1), 65-76.

110. Lebrusant-Fernandez, M.; ap Rees, T.; Jimeno, R.; Angelis, N.; Ng, J. C.; Fraternali, F.; Li, V. S. W.; Barral, P., IFN-γ-dependent regulation of intestinal epithelial homeostasis by NKT cells. *Cell Reports* **2024,** 43, (12), 114948.

111. Glading, A.; Lauffenburger, D. A.; Wells, A., Cutting to the chase: calpain proteases in cell motility. *Trends Cell Biol* **2002,** 12, (1), 46-54.

112. Wells, A.; Huttenlocher, A.; Lauffenburger, D. A., Calpain proteases in cell adhesion and motility. *Int Rev Cytol* **2005,** 245, 1-16.

113. Franco, S. J.; Huttenlocher, A., Regulating cell migration: calpains make the cut. *J Cell Sci* **2005,** 118, (Pt 17), 3829-38.

114. Chun, J.; Prince, A., Ca2+ signaling in airway epithelial cells facilitates leukocyte recruitment and transepithelial migration. *J Leukoc Biol* **2009,** 86, (5), 1135-44.

115. Cario, E.; Gerken, G.; Podolsky, D. K., Toll-Like Receptor 2 Controls Mucosal Inflammation by Regulating Epithelial Barrier Function. *Gastroenterology* **2007,** 132, (4), 1359-1374.

116. Soong, G.; Chun, J.; Parker, D.; Prince, A., Staphylococcus aureus Activation of Caspase 1/Calpain Signaling Mediates Invasion Through Human Keratinocytes. *The Journal of Infectious Diseases* **2012,** 205, (10), 1571-1579.

117. Mai, J.; Virtue, A.; Shen, J.; Wang, H.; Yang, X.-F., An evolving new paradigm: endothelial cells – conditional innate immune cells. *Journal of Hematology & Oncology* **2013,** 6, (1), 61.

118. Einhorn, S.; Eldor, A.; Vlodavsky, I.; Fuks, Z.; Panet, A., Production and characterization of interferon from endothelial cells. *J Cell Physiol* **1985,** 122, (2), 200-4.

119. Lu, Y.; Sun, Y.; Xu, K.; Shao, Y.; Saaoud, F.; Snyder, N. W.; Yang, L.; Yu, J.; Wu, S.; Hu, W.; Sun, J.; Wang, H.; Yang, X., Editorial: Endothelial cells as innate immune cells. *Front Immunol* **2022,** 13, 1035497.

120. Chang, R. T.; Fisher, M. J.; Sumbria, R. K., Brain endothelial cells as phagocytes: mechanisms and implications. *Fluids Barriers CNS* **2025,** 22, (1), 30.

121. Amersfoort, J.; Eelen, G.; Carmeliet, P., Immunomodulation by endothelial cells — partnering up with the immune system? *Nature Reviews Immunology* **2022,** 22, (9), 576-588.

122. Shao, Y.; Saredy, J.; Yang, W. Y.; Sun, Y.; Lu, Y.; Saaoud, F.; Drummer, C.; Johnson, C.; Xu, K.; Jiang, X.; Wang, H.; Yang, X., Vascular Endothelial Cells and Innate Immunity. *Arteriosclerosis, Thrombosis, and Vascular Biology* **2020,** 40, (6), e138-e152.

123. Zhang, Y.; Liu, N. M.; Wang, Y.; Youn, J. Y.; Cai, H., Endothelial cell calpain as a critical modulator of angiogenesis. *Biochim Biophys Acta Mol Basis Dis* **2017,** 1863, (6), 1326-1335.

124. Miyazaki, T.; Akasu, R.; Miyazaki, A., Calpain proteolytic systems counteract endothelial cell adaptation to inflammatory environments. *Inflammation and Regeneration* **2020,** 40, (1), 5.

125. Youn, J.-Y.; Wang, T.; Cai, H., An Ezrin/Calpain/PI3K/AMPK/eNOSs1179 Signaling Cascade Mediating VEGF-Dependent Endothelial Nitric Oxide Production. *Circulation Research* **2009,** 104, (1), 50-59.

126. Bodnar, R. J.; Yates, C. C.; Wells, A., IP-10 Blocks Vascular Endothelial Growth Factor-Induced Endothelial Cell Motility and Tube Formation via Inhibition of Calpain. *Circulation Research* **2006,** 98, (5), 617-625.

127. Su, Y.; Cui, Z.; Li, Z.; Block, E. R., Calpain-2 regulation of VEGF-mediated angiogenesis. *The FASEB Journal* **2006,** 20, (9), 1443-1451.

128. Zhang, Y.; Li, Q.; Youn, J. Y.; Cai, H., Protein Phosphotyrosine Phosphatase 1B (PTP1B) in Calpain-dependent Feedback Regulation of Vascular Endothelial Growth Factor Receptor (VEGFR2) in Endothelial Cells: IMPLICATIONS IN VEGF-DEPENDENT ANGIOGENESIS AND DIABETIC WOUND HEALING*. *Journal of Biological Chemistry* **2017,** 292, (2), 407-416.

129. Liu, W.; Ma, K.; Kwon, S. H.; Garg, R.; Patta, Y. R.; Fujiwara, T.; Gurtner, G. C., The Abnormal Architecture of Healed Diabetic Ulcers Is the Result of FAK Degradation by Calpain 1. *J Invest Dermatol* **2017,** 137, (5), 1155-1165.

130. Yi, C.; Wu, W.; Zheng, D.; Peng, G.; Huang, H.; Shen, Z.; Teng, X., Targeted inhibition of endothelial calpain delays wound healing by reducing inflammation and angiogenesis. *Cell Death & Disease* **2020,** 11, (7), 533.

131. Rembe, J.-D.; Garabet, W.; Augustin, M.; Dissemond, J.; Ibing, W.; Schelzig, H.; Stuermer, E. K., Immunomarker profiling in human chronic wound swabs reveals IL-1 beta/IL-1RA and CXCL8/CXCL10 ratios as potential biomarkers for wound healing, infection status and regenerative stage. *Journal of Translational Medicine* **2025,** 23, (1), 407.

132. Nawaz, M. I.; Van Raemdonck, K.; Mohammad, G.; Kangave, D.; Van Damme, J.; Abu El-Asrar, A. M.; Struyf, S., Autocrine CCL2, CXCL4, CXCL9 and CXCL10 signal in retinal endothelial cells and are enhanced in diabetic retinopathy. *Experimental Eye Research* **2013,** 109, 67-76.

133. Zhang, Y.; Thai, K.; Kepecs, D. M.; Winer, D.; Gilbert, R. E., Reversing CXCL10 Deficiency Ameliorates Kidney Disease in Diabetic Mice. *The American Journal of Pathology* **2018,** 188, (12), 2763-2773.

134. Potz, B. A.; Sabe, A. A.; Sabe, S. A.; Lawandy, I. J.; Abid, M. R.; Clements, R. T.; Sellke, F. W., Calpain inhibition decreases myocardial fibrosis in chronically ischemic hypercholesterolemic swine. *The Journal of Thoracic and Cardiovascular Surgery* **2022,** 163, (1), e11-e27.

135. Miyazaki, T.; Taketomi, Y.; Saito, Y.; Hosono, T.; Lei, X.-F.; Kim-Kaneyama, J.-r.; Arata, S.; Takahashi, H.; Murakami, M.; Miyazaki, A., Calpastatin Counteracts Pathological Angiogenesis by Inhibiting Suppressor of Cytokine Signaling 3 Degradation in Vascular Endothelial Cells. *Circulation Research* **2015,** 116, (7), 1170-1181.

136. Gariano, R. F.; Gardner, T. W., Retinal angiogenesis in development and disease. *Nature* **2005,** 438, (7070), 960-6.

137. Carmeliet, P.; Jain, R. K., Molecular mechanisms and clinical applications of angiogenesis. *Nature* **2011,** 473, (7347), 298-307.

138. Zhang, J.; Patel, J. M.; Block, E. R., Hypoxia-specific upregulation of calpain activity and gene expression in pulmonary artery endothelial cells. *American Journal of Physiology-Lung Cellular and Molecular Physiology* **1998,** 275, (3), L461-L468.

139. Aono, Y.; Ariyoshi, H.; Tsuji, Y.; Ueda, A.; Tokunaga, M.; Sakon, M.; Monden, M., Localized Activation of m-Calpain in Human Umbilical Vein Endothelial Cells Upon Hypoxia. *Thrombosis Research* **2001,** 102, (4), 353-361.

140. Mo, X.-G.; Chen, Q.-W.; Li, X.-S.; Zheng, M.-M.; Ke, D.-Z.; Deng, W.; Li, G.-Q.; Jiang, J.; Wu, Z.-Q.; Wang, L.; Wang, P.; Yang, Y.; Cao, G.-Y., Suppression of NHE1 by small interfering RNA inhibits HIF-1α-induced angiogenesis in vitro via modulation of calpain activity. *Microvascular Research* **2011,** 81, (2), 160-168.

141. Sanson, M.; Ingueneau, C.; Vindis, C.; Thiers, J. C.; Glock, Y.; Rousseau, H.; Sawa, Y.; Bando, Y.; Mallat, Z.; Salvayre, R.; Nègre-Salvayre, A., Oxygen-regulated protein-150 prevents calcium homeostasis deregulation and apoptosis induced by oxidized LDL in vascular cells. *Cell Death & Differentiation* **2008,** 15, (8), 1255-1265.

142. Tsimikas, S.; Witztum, J. L., Oxidized phospholipids in cardiovascular disease. *Nature Reviews Cardiology* **2024,** 21, (3), 170-191.

143. Vindis, C.; Elbaz, M.; Escargueil-Blanc, I.; Augé, N.; Heniquez, A.; Thiers, J.-C.; Nègre-Salvayre, A.; Salvayre, R., Two Distinct Calcium-Dependent Mitochondrial Pathways Are Involved in Oxidized LDL-Induced Apoptosis. *Arteriosclerosis, Thrombosis, and Vascular Biology* **2005,** 25, (3), 639-645.

144. Gonçalves, I.; Nitulescu, M.; Saido, T. C.; Dias, N.; Pedro, L. M.; e Fernandes, J. F.; Ares, M. P. S.; Pörn-Ares, I., Activation of calpain-1 in human carotid artery atherosclerotic lesions. *BMC Cardiovascular Disorders* **2009,** 9, (1), 26.

145. Chen, Z.; Xue, Q.; Cao, L.; Wang, Y.; Chen, Y.; Zhang, X.; Xiao, F.; Yang, Y.; Hayden, M. R.; Liu, Y.; Yang, K., Toll-Like Receptor 4 Mediated Oxidized Low-Density Lipoprotein-Induced Foam Cell Formation in Vascular Smooth Muscle Cells via Src and Sirt1/3 Pathway. *Mediators Inflamm* **2021,** 2021, 6639252.

146. Miyazaki, T.; Taketomi, Y.; Takimoto, M.; Lei, X.-F.; Arita, S.; Kim-Kaneyama, J.-r.; Arata, S.; Ohata, H.; Ota, H.; Murakami, M.; Miyazaki, A., m-Calpain Induction in Vascular Endothelial Cells on Human and Mouse Atheromas and Its Roles in VE-Cadherin Disorganization and Atherosclerosis. *Circulation* **2011,** 124, (23), 2522-2532.

147. Jiang, J.; Hiron, T. K.; Chalisey, A.; Malhotra, Y.; Agbaedeng, T.; O'Callaghan, C. A., Ox-LDL induces a non-inflammatory response enriched for coronary artery disease risk in human endothelial cells. *Scientific Reports* **2025,** 15, (1), 21877.

148. Miyazaki, T.; Taketomi, Y.; Higashi, T.; Ohtaki, H.; Takaki, T.; Ohnishi, K.; Hosonuma, M.; Kono, N.; Akasu, R.; Haraguchi, S.; Kim-Kaneyama, J.-R.; Otsu, K.; Arai, H.; Murakami, M.; Miyazaki, A., Hypercholesterolemic Dysregulation of Calpain in Lymphatic Endothelial Cells Interferes With Regulatory T-Cell Stability and Trafficking. *Arteriosclerosis, Thrombosis, and Vascular Biology* **2023,** 43, (2), e66-e82.

149. Shi, Y.; Gu, W.; Wei, Y.; Li, S.; Zhang, S.; Jiang, Y.; Chen, C.; Liu, T.; Shuai, L.; Zhou, X.; Tang, F., Deficiency of Calpain-1 attenuates atherosclerotic plaque and calcification and improves vasomotor dysfunction in Apolipoprotein E knockout mice through inhibiting inflammation. *Biochem Biophys Res Commun* **2025,** 749, 151369.

150. Liu, Z.; Ji, J.; Zheng, D.; Su, L.; Peng, T.; Tang, J., Protective role of endothelial calpain knockout in lipopolysaccharide-induced acute kidney injury via attenuation of the p38-iNOS pathway and NO/ROS production. *Experimental & Molecular Medicine* **2020,** 52, (4), 702-712.

151. Alluri, H.; Grimsley, M.; Anasooya Shaji, C.; Varghese, K. P.; Zhang, S. L.; Peddaboina, C.; Robinson, B.; Beeram, M. R.; Huang, J. H.; Tharakan, B., Attenuation of Blood-Brain Barrier Breakdown and Hyperpermeability by Calpain Inhibition. *J Biol Chem* **2016,** 291, (53), 26958-26969.

152. Puerta-Guardo, H.; Biering, S. B.; Castillo-Rojas, B.; DiBiasio-White, M. J.; Lo, N. T.; Espinosa, D. A.; Warnes, C. M.; Wang, C.; Cao, T.; Glasner, D. R.; Beatty, P. R.; Kuhn, R. J.; Harris, E., Flavivirus NS1-triggered endothelial dysfunction promotes virus dissemination. *bioRxiv* **2024**.

153. Li, J.; Zheng, K.; Shen, H.; Wu, H.; Wan, C.; Zhang, R.; Liu, Z., Calpain-2 protein influences chikungunya virus replication and regulates vimentin rearrangement caused by chikungunya virus infection. *Frontiers in Microbiology* **2023,** Volume 14 - 2023.

154. Mackow, E. R.; Gorbunova, E. E.; Gavrilovskaya, I. N., Endothelial cell dysfunction in viral hemorrhage and edema. *Front Microbiol* **2014,** 5, 733.

155. Ghosh Roy, S.; Sadigh, B.; Datan, E.; Lockshin, R. A.; Zakeri, Z., Regulation of cell survival and death during Flavivirus infections. *World J Biol Chem* **2014,** 5, (2), 93-105.

156. Hussain, A. M.; Zhang, Q. X.; Murray, A. G., Endothelial Cell Calpain Activity Facilitates Lymphocyte Diapedesis. *American Journal of Transplantation* **2005,** 5, (11), 2640-2648.

157. Filippi, M. D., Mechanism of Diapedesis: Importance of the Transcellular Route. *Adv Immunol* **2016,** 129, 25-53.

158. Muller, W. A., Mechanisms of Transendothelial Migration of Leukocytes. *Circulation Research* **2009,** 105, (3), 223-230.

159. Etwebi, Z.; Landesberg, G.; Preston, K.; Eguchi, S.; Scalia, R., Mechanistic Role of the Calcium-Dependent Protease Calpain in the Endothelial Dysfunction Induced by MPO (Myeloperoxidase). *Hypertension* **2018,** 71, (4), 761-770.

160. Kumar, V.; Sharma, A., Mast cells: emerging sentinel innate immune cells with diverse role in immunity. *Mol Immunol* **2010,** 48, (1-3), 14-25.

161. Kumar, V.; Sharma, A., Neutrophils: Cinderella of innate immune system. *Int Immunopharmacol* **2010,** 10, (11), 1325-34.

162. Kumar, V., Dendritic cells in sepsis: Potential immunoregulatory cells with therapeutic potential. *Mol Immunol* **2018,** 101, 615-626.

163. Takano, E.; Park, Y. H.; Kitahara, A.; Yamagata, Y.; Kannagi, R.; Murachi, T., Distribution of calpains and calpastatin in human blood cells. *Biochem Int* **1988,** 16, (3), 391-5.

164. Huang, Z.; Hoffmann, F. W.; Norton, R. L.; Hashimoto, A. C.; Hoffmann, P. R., Selenoprotein K Is a Novel Target of m-Calpain, and Cleavage Is Regulated by Toll-like Receptor-induced Calpastatin in Macrophages *. *Journal of Biological Chemistry* **2011,** 286, (40), 34830-34838.

165. Ma, J.; Kummarapurugu, A. B.; Zheng, S.; Ghio, A. J.; Deshpande, L. S.; Voynow, J. A., Neutrophil elastase activates macrophage calpain as a mechanism for phagocytic failure. *American Journal of Physiology-Lung Cellular and Molecular Physiology* **2025,** 328, (1), L93-L104.

166. Mass, E.; Nimmerjahn, F.; Kierdorf, K.; Schlitzer, A., Tissue-specific macrophages: how they develop and choreograph tissue biology. *Nat Rev Immunol* **2023,** 23, (9), 563-579.

167. Vijayan, V.; Pradhan, P.; Braud, L.; Fuchs, H. R.; Gueler, F.; Motterlini, R.; Foresti, R.; Immenschuh, S., Human and murine macrophages exhibit differential metabolic responses to lipopolysaccharide - A divergent role for glycolysis. *Redox Biol* **2019,** 22, 101147.

168. Schneemann, M.; Schoedon, G., Species differences in macrophage NO production are important. *Nature Immunology* **2002,** 3, (2), 102-102.

169. Guan, F.; Wang, R.; Yi, Z.; Luo, P.; Liu, W.; Xie, Y.; Liu, Z.; Xia, Z.; Zhang, H.; Cheng, Q., Tissue macrophages: origin, heterogenity, biological functions, diseases and therapeutic targets. *Signal Transduction and Targeted Therapy* **2025,** 10, (1), 93.

170. Bruscia, E. M.; Zhang, P. X.; Satoh, A.; Caputo, C.; Medzhitov, R.; Shenoy, A.; Egan, M. E.; Krause, D. S., Abnormal trafficking and degradation of TLR4 underlie the elevated inflammatory response in cystic fibrosis. *J Immunol* **2011,** 186, (12), 6990-8.

171. Hofer, T. P.; Frankenberger, M.; Heimbeck, I.; Burggraf, D.; Wjst, M.; Wright, A. K.; Kerscher, M.; Nährig, S.; Huber, R. M.; Fischer, R.; Ziegler-Heitbrock, L., Decreased expression of HLA-DQ and HLA-DR on cells of the monocytic lineage in cystic fibrosis. *J Mol Med (Berl)* **2014,** 92, (12), 1293-304.

172. Alexis, N. E.; Muhlebach, M. S.; Peden, D. B.; Noah, T. L., Attenuation of host defense function of lung phagocytes in young cystic fibrosis patients. *J Cyst Fibros* **2006,** 5, (1), 17-25.

173. Jaganathan, D.; Bruscia, E. M.; Kopp, B. T., Emerging Concepts in Defective Macrophage Phagocytosis in Cystic Fibrosis. *Int J Mol Sci* **2022,** 23, (14).

174. Fettucciari, K.; Quotadamo, F.; Noce, R.; Palumbo, C.; Modesti, A.; Rosati, E.; Mannucci, R.; Bartoli, A.; Marconi, P., Group B Streptococcus (GBS) disrupts by calpain activation the actin and microtubule cytoskeleton of macrophages. *Cell Microbiol* **2011,** 13, (6), 859-84.

175. Fettucciari, K.; Fetriconi, I.; Mannucci, R.; Nicoletti, I.; Bartoli, A.; Coaccioli, S.; Marconi, P., Group B Streptococcus induces macrophage apoptosis by calpain activation. *J Immunol* **2006,** 176, (12), 7542-56.

176. Liu, X.; Van Vleet, T.; Schnellmann, R. G., The role of calpain in oncotic cell death. *Annu Rev Pharmacol Toxicol* **2004,** 44, 349-70.

177. Goldmann, O.; Sastalla, I.; Wos-Oxley, M.; Rohde, M.; Medina, E., Streptococcus pyogenes induces oncosis in macrophages through the activation of an inflammatory programmed cell death pathway. *Cell Microbiol* **2009,** 11, (1), 138-55.

178. Ulett, G. C.; Maclean, K. H.; Nekkalapu, S.; Cleveland, J. L.; Adderson, E. E., Mechanisms of group B streptococcal-induced apoptosis of murine macrophages. *J Immunol* **2005,** 175, (4), 2555-62.

179. De-Leon-Lopez, Y. S.; Thompson, M. E.; Kean, J. J.; Flaherty, R. A., The PI3K-Akt pathway is a multifaceted regulator of the macrophage response to diverse group B Streptococcus isolates. *Front Cell Infect Microbiol* **2023,** 13, 1258275.

180. Lopez-Castejon, G.; Corbett, D.; Goldrick, M.; Roberts, I. S.; Brough, D., Inhibition of Calpain Blocks the Phagosomal Escape of Listeria monocytogenes. *PLOS ONE* **2012,** 7, (4), e35936.

181. Dewamitta, S. R.; Nomura, T.; Kawamura, I.; Hara, H.; Tsuchiya, K.; Kurenuma, T.; Shen, Y.; Daim, S.; Yamamoto, T.; Qu, H.; Sakai, S.; Xu, Y.; Mitsuyama, M., Listeriolysin O-Dependent Bacterial Entry into the Cytoplasm Is Required for Calpain Activation and Interleukin-1αSecretion in Macrophages Infected with <i>Listeria monocytogenes</i>. *Infection and Immunity* **2010,** 78, (5), 1884-1894.

182. Yao, B.; Wang, J.; Hou, D.; Liu, W.; Xie, C.; Shi, K.; Han, W.; Miao, X.; Chen, J.; Cai, Z.; Yang, H.; Ling, Q.; Yin, K.; Dong, Z.; Huang, Z., Calpain-2 facilitates infection of the intracellular bacteria Listeria monocytogenes and invasion intestinal immune barrier by impairing nitric oxide homeostasis. *Journal of Advanced Research* **2025.**

183. Bonnardel, J.; Da Silva, C.; Henri, S.; Tamoutounour, S.; Chasson, L.; Montañana-Sanchis, F.; Gorvel, J.-P.; Lelouard, H., Innate and Adaptive Immune Functions of Peyer’s Patch Monocyte-Derived Cells. *Cell Reports* **2015,** 11, (5), 770-784.

184. Jung, C.; Hugot, J. P.; Barreau, F., Peyer's Patches: The Immune Sensors of the Intestine. *Int J Inflam* **2010,** 2010, 823710.

185. Lim, Y. J.; Choi, H. H.; Choi, J. A.; Jeong, J. A.; Cho, S. N.; Lee, J. H.; Park, J. B.; Kim, H. J.; Song, C. H., Mycobacterium kansasii-induced death of murine macrophages involves endoplasmic reticulum stress responses mediated by reactive oxygen species generation or calpain activation. *Apoptosis* **2013,** 18, (2), 150-9.

186. Castillo, E. F.; Dekonenko, A.; Arko-Mensah, J.; Mandell, M. A.; Dupont, N.; Jiang, S.; Delgado-Vargas, M.; Timmins, G. S.; Bhattacharya, D.; Yang, H.; Hutt, J.; Lyons, C. R.; Dobos, K. M.; Deretic, V., Autophagy protects against active tuberculosis by suppressing bacterial burden and inflammation. *Proc Natl Acad Sci U S A* **2012,** 109, (46), E3168-76.

187. Russo, R.; Berliocchi, L.; Adornetto, A.; Varano, G. P.; Cavaliere, F.; Nucci, C.; Rotiroti, D.; Morrone, L. A.; Bagetta, G.; Corasaniti, M. T., Calpain-mediated cleavage of Beclin-1 and autophagy deregulation following retinal ischemic injury in vivo. *Cell Death Dis* **2011,** 2, (4), e144.

188. Ahn, D.; Peñaloza, H.; Wang, Z.; Wickersham, M.; Parker, D.; Patel, P.; Koller, A.; Chen, E. I.; Bueno, S. M.; Uhlemann, A. C.; Prince, A., Acquired resistance to innate immune clearance promotes Klebsiella pneumoniae ST258 pulmonary infection. *JCI Insight* **2016,** 1, (17), e89704.

189. Lee, F. Y.; Kim, D. W.; Karmin, J. A.; Hong, D.; Chang, S. S.; Fujisawa, M.; Takayanagi, H.; Bigliani, L. U.; Blaine, T. A.; Lee, H. J., mu-Calpain regulates receptor activator of NF-kappaB ligand (RANKL)-supported osteoclastogenesis via NF-kappaB activation in RAW 264.7 cells. *J Biol Chem* **2005,** 280, (33), 29929-36.

190. Huang, R.; Wang, X.; Zhou, Y.; Xiao, Y., RANKL-induced M1 macrophages are involved in bone formation. *Bone Research* **2017,** 5, (1), 17019.

191. Ahern, E.; Smyth, M. J.; Dougall, W. C.; Teng, M. W. L., Roles of the RANKL–RANK axis in antitumour immunity — implications for therapy. *Nature Reviews Clinical Oncology* **2018,** 15, (11), 676-693.

192. Rigoni, T. S.; Vellozo, N. S.; Cabral-Piccin, M.; Fabiano-Coelho, L.; Lopes, U. G.; Filardy, A. A.; DosReis, G. A.; Lopes, M. F., RANK Ligand Helps Immunity to Leishmania major by Skewing M2-Like Into M1 Macrophages. *Frontiers in Immunology* **2020,** Volume 11 - 2020.

193. Zhang, L.; Zheng, D.; Yan, Y.; Yu, Y.; Chen, R.; Li, Z.; Greer, P. A.; Peng, T.; Wang, Q., Myeloid cell-specific deletion of Capns1 prevents macrophage polarization toward the M1 phenotype and reduces interstitial lung disease in the bleomycin model of systemic sclerosis. *Arthritis Research & Therapy* **2022,** 24, (1), 148.

194. Zhang, G.; Thomas, A. L.; Marshall, A. L.; Kernan, K. A.; Su, Y.; Zheng, Y.; Takano, J.; Saido, T. C.; Eddy, A. A., Nicotinic acetylcholine receptor α1 promotes calpain-1 activation and macrophage inflammation in hypercholesterolemic nephropathy. *Lab Invest* **2011,** 91, (1), 106-23.

195. Kumar, V.; Sharma, A., Is neuroimmunomodulation a future therapeutic approach for sepsis? *International Immunopharmacology* **2010,** 10, (1), 9-17.

196. Hone, A. J.; McIntosh, J. M., Nicotinic acetylcholine receptors: Therapeutic targets for novel ligands to treat pain and inflammation. *Pharmacological Research* **2023,** 190, 106715.

197. Papke, R. L.; Lindstrom, J. M., Nicotinic acetylcholine receptors: Conventional and unconventional ligands and signaling. *Neuropharmacology* **2020,** 168, 108021.

198. Mikulski, Z.; Hartmann, P.; Jositsch, G.; Zasłona, Z.; Lips, K. S.; Pfeil, U.; Kurzen, H.; Lohmeyer, J.; Clauss, W. G.; Grau, V.; Fronius, M.; Kummer, W., Nicotinic receptors on rat alveolar macrophages dampen ATP-induced increase in cytosolic calcium concentration. *Respiratory Research* **2010,** 11, (1), 133.

199. Cauwels, A.; Rogge, E.; Vandendriessche, B.; Shiva, S.; Brouckaert, P., Extracellular ATP drives systemic inflammation, tissue damage and mortality. *Cell Death & Disease* **2014,** 5, (3), e1102-e1102.

200. Di Virgilio, F.; Vultaggio-Poma, V.; Falzoni, S.; Giuliani, A. L., Extracellular ATP: A powerful inflammatory mediator in the central nervous system. *Neuropharmacology* **2023,** 224, 109333.

201. Savio, L. E. B., P2X receptors in the balance between inflammation and pathogen control in sepsis. *Purinergic Signalling* **2022,** 18, (3), 241-243.

202. Välimäki, E.; Cypryk, W.; Virkanen, J.; Nurmi, K.; Turunen, P. M.; Eklund, K. K.; Åkerman, K. E.; Nyman, T. A.; Matikainen, S., Calpain Activity Is Essential for ATP-Driven Unconventional Vesicle-Mediated Protein Secretion and Inflammasome Activation in Human Macrophages. *The Journal of Immunology* **2016,** 197, (8), 3315-3325.

203. Wan, F.; Letavernier, E.; Le Saux, C. J.; Houssaini, A.; Abid, S.; Czibik, G.; Sawaki, D.; Marcos, E.; Dubois-Rande, J. L.; Baud, L.; Adnot, S.; Derumeaux, G.; Gellen, B., Calpastatin overexpression impairs postinfarct scar healing in mice by compromising reparative immune cell recruitment and activation. *Am J Physiol Heart Circ Physiol* **2015,** 309, (11), H1883-93.

204. Kumar, V.; Stewart IV, J. H., Obesity, bone marrow adiposity, and leukemia: Time to act. *Obesity Reviews* **2024,** 25, (3), e13674.

205. Kumar, V.; Kiran, S.; Kumar, S.; Singh, U. P., Extracellular vesicles in obesity and its associated inflammation. *International Reviews of Immunology* **2022,** 41, (1), 30-44.

206. Chakarov, S.; Blériot, C.; Ginhoux, F., Role of adipose tissue macrophages in obesity-related disorders. *Journal of Experimental Medicine* **2022,** 219, (7).

207. Weisberg, S. P.; McCann, D.; Desai, M.; Rosenbaum, M.; Leibel, R. L.; Ferrante, A. W., Jr., Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* **2003,** 112, (12), 1796-808.

208. Miyazaki, T.; Miyazaki, A., Emerging roles of calpain proteolytic systems in macrophage cholesterol handling. *Cell Mol Life Sci* **2017,** 74, (16), 3011-3021.

209. Thrum, S.; Sommer, M.; Raulien, N.; Gericke, M.; Massier, L.; Kovacs, P.; Krasselt, M.; Landgraf, K.; Körner, A.; Dietrich, A.; Blüher, M.; Rossol, M.; Wagner, U., Macrophages in obesity are characterised by increased IL-1β response to calcium-sensing receptor signals. *International Journal of Obesity* **2022,** 46, (10), 1883-1891.

210. Xu, H.; Zhang, L.; Xu, D.; Deng, W.; Yang, W.; Tang, F.; Da, M., Knockout of calpain-1 protects against high-fat diet-induced liver dysfunction in mouse through inhibiting oxidative stress and inflammation. *Food Sci Nutr* **2021,** 9, (1), 367-374.

211. Awad, M. A.; Gu, W.; Li, S.; Wei, Y.; Tang, Y.; Shi, Y.; Juvenal, H.; Jiang, Y.; Liu, T.; Shuai, L.; Wang, Z.; Wu, B.; Zhou, X.; Tang, F., Calpain-1 is required for abnormality of liver function and metabolism in apolipoprotein E knockout mouse evaluated noninvasively by small animal MRI and PET-CT. *Biochim Biophys Acta Mol Cell Biol Lipids* **2025,** 1870, (5), 159616.

212. Muniappan, L.; Javidan, A.; Jiang, W.; Mohammadmoradi, S.; Moorleghen, J. J.; Katz, W. S.; Balakrishnan, A.; Howatt, D. A.; Subramanian, V., Calpain Inhibition Attenuates Adipose Tissue Inflammation and Fibrosis in Diet-induced Obese Mice. *Scientific Reports* **2017,** 7, (1), 14398.

213. Subramanian, V.; Balakrishnan, A.; Howatt, D. A.; Moorleghen, J. J.; Katz, W. S., Abstract 81: Pharmacological Inhibition of Calpain Attenuates Adipose Tissue Apoptosis, Macrophage Accumulation and Inflammation in Diet-induced Obese Mice. *Arteriosclerosis, Thrombosis, and Vascular Biology* **2013,** 33, (suppl_1), A81-A81.

214. Hanouna, G.; Mesnard, L.; Vandermeersch, S.; Perez, J.; Placier, S.; Haymann, J.-P.; Campagne, F.; Moroch, J.; Bataille, A.; Baud, L.; Letavernier, E., Specific calpain inhibition protects kidney against inflammaging. *Scientific Reports* **2017,** 7, (1), 8016.

215. Lokuta, M. A.; Nuzzi, P. A.; Huttenlocher, A., Calpain regulates neutrophil chemotaxis. *Proc Natl Acad Sci U S A* **2003,** 100, (7), 4006-11.

216. Katsube, M.; Kato, T.; Kitagawa, M.; Noma, H.; Fujita, H.; Kitagawa, S., Calpain-mediated regulation of the distinct signaling pathways and cell migration in human neutrophils. *J Leukoc Biol* **2008,** 84, (1), 255-63.

217. Knepper-Nicolai, B.; Savill, J.; Brown, S. B., Constitutive Apoptosis in Human Neutrophils Requires Synergy between Calpains and the Proteasome Downstream of Caspases *. *Journal of Biological Chemistry* **1998,** 273, (46), 30530-30536.

218. Ortega-Gómez, A.; Perretti, M.; Soehnlein, O., Resolution of inflammation: an integrated view. *EMBO Molecular Medicine* **2013,** 5, (5), 661-674.

219. Lawrence, T.; Gilroy, D. W., Chronic inflammation: a failure of resolution? *Int J Exp Pathol* **2007,** 88, (2), 85-94.

220. Jundi, B.; Lee, D.-H.; Jeon, H.; Duvall, M. G.; Nijmeh, J.; Abdulnour, R.-E. E.; Pinilla-Vera, M.; Baron, R. M.; Han, J.; Voldman, J.; Levy, B. D., Inflammation resolution circuits are uncoupled in acute sepsis and correlate with clinical severity. *JCI Insight* **2021,** 6, (15).

221. Delano, M. J.; Ward, P. A., The immune system's role in sepsis progression, resolution, and long-term outcome. *Immunol Rev* **2016,** 274, (1), 330-353.

222. Jennings, S.; Hallett, M. B., Single cell measurement of calpain activity in neutrophils reveals link to cytosolic Ca2+ elevation and individual phagocytotic events. *Biochemical and Biophysical Research Communications* **2019,** 515, (1), 163-168.

223. Dewitt, S.; Francis, R. J.; Hallett, M. B., Ca2+ and calpain control membrane expansion during the rapid cell spreading of neutrophils. *Journal of Cell Science* **2013,** 126, (20), 4627-4635.

224. Singh, J.; Zlatar, L.; Muñoz-Becerra, M.; Lochnit, G.; Herrmann, I.; Pfister, F.; Janko, C.; Knopf, J.; Leppkes, M.; Schoen, J.; Muñoz, L. E.; Schett, G.; Herrmann, M.; Schauer, C.; Mahajan, A., Calpain-1 weakens the nuclear envelope and promotes the release of neutrophil extracellular traps. *Cell Communication and Signaling* **2024,** 22, (1), 435.

225. Gößwein, S.; Lindemann, A.; Mahajan, A.; Maueröder, C.; Martini, E.; Patankar, J.; Schett, G.; Becker, C.; Wirtz, S.; Naumann-Bartsch, N.; Bianchi, M. E.; Greer, P. A.; Lochnit, G.; Herrmann, M.; Neurath, M. F.; Leppkes, M., Citrullination Licenses Calpain to Decondense Nuclei in Neutrophil Extracellular Trap Formation. *Front Immunol* **2019,** 10, 2481.

226. Sørensen, O. E.; Borregaard, N., Neutrophil extracellular traps - the dark side of neutrophils. *J Clin Invest* **2016,** 126, (5), 1612-20.

227. Baratchi, S.; Danish, H.; Chheang, C.; Zhou, Y.; Huang, A.; Lai, A.; Khanmohammadi, M.; Quinn, K. M.; Khoshmanesh, K.; Peter, K., Piezo1 expression in neutrophils regulates shear-induced NETosis. *Nature Communications* **2024,** 15, (1), 7023.

228. Wang, H.; Kim, S. J.; Lei, Y.; Wang, S.; Wang, H.; Huang, H.; Zhang, H.; Tsung, A., Neutrophil extracellular traps in homeostasis and disease. *Signal Transduction and Targeted Therapy* **2024,** 9, (1), 235.

229. Wiemer, A. J.; Lokuta, M. A.; Surfus, J. C.; Wernimont, S. A.; Huttenlocher, A., Calpain inhibition impairs TNF-alpha-mediated neutrophil adhesion, arrest and oxidative burst. *Mol Immunol* **2010,** 47, (4), 894-902.

230. Wiemer, A. J.; Lokuta, M. A.; Surfus, J. C.; Wernimont, S. A.; Huttenlocher, A., Calpain inhibition impairs TNF-α-mediated neutrophil adhesion, arrest and oxidative burst. *Molecular Immunology* **2010,** 47, (4), 894-902.

231. Moser, M., Dendritic Cells in Immunity and Tolerance—Do They Display Opposite Functions? *Immunity* **2003,** 19, (1), 5-8.

232. Heras-Murillo, I.; Adán-Barrientos, I.; Galán, M.; Wculek, S. K.; Sancho, D., Dendritic cells as orchestrators of anticancer immunity and immunotherapy. *Nature Reviews Clinical Oncology* **2024,** 21, (4), 257-277.

233. Moon, C. Y.; Belabed, M.; Park, M. D.; Mattiuz, R.; Puleston, D.; Merad, M., Dendritic cell maturation in cancer. *Nature Reviews Cancer* **2025,** 25, (4), 225-248.

234. Calle, Y.; Carragher, N. O.; Thrasher, A. J.; Jones, G. E., Inhibition of calpain stabilises podosomes and impairs dendritic cell motility. *Journal of Cell Science* **2006,** 119, (11), 2375-2385.

235. Shumilina, E.; Huber, S. M.; Lang, F., Ca2+ signaling in the regulation of dendritic cell functions. *American Journal of Physiology-Cell Physiology* **2011,** 300, (6), C1205-C1214.

236. Calle, Y.; Burns, S.; Thrasher, A. J.; Jones, G. E., The leukocyte podosome. *Eur J Cell Biol* **2006,** 85, (3-4), 151-7.

237. Hidalgo, A.; Frenette, P. S., Leukocyte Podosomes Sense Their Way through the Endothelium. *Immunity* **2007,** 26, (6), 753-755.

238. Carman, C. V.; Sage, P. T.; Sciuto, T. E.; de la Fuente, M. A.; Geha, R. S.; Ochs, Hans D.; Dvorak, H. F.; Dvorak, A. M.; Springer, T. A., Transcellular Diapedesis Is Initiated by Invasive Podosomes. *Immunity* **2007,** 26, (6), 784-797.

239. Zhang, J.; Zahir, N.; Jiang, Q.; Miliotis, H.; Heyraud, S.; Meng, X.; Dong, B.; Xie, G.; Qiu, F.; Hao, Z.; McCulloch, C. A.; Keystone, E. C.; Peterson, A. C.; Siminovitch, K. A., The autoimmune disease–associated PTPN22 variant promotes calpain-mediated Lyp/Pep degradation associated with lymphocyte and dendritic cell hyperresponsiveness. *Nature Genetics* **2011,** 43, (9), 902-907.

240. Behrens, T. W., Lyp breakdown and autoimmunity. *Nature Genetics* **2011,** 43, (9), 821-822.

241. Tizaoui, K.; Terrazzino, S.; Cargnin, S.; Lee, K. H.; Gauckler, P.; Li, H.; Shin, J. I.; Kronbichler, A., The role of PTPN22 in the pathogenesis of autoimmune diseases: A comprehensive review. *Semin Arthritis Rheum* **2021,** 51, (3), 513-522.

242. Hamel-Côté, G.; Gendron, D.; Rola-Pleszczynski, M.; Stankova, J., Regulation of platelet-activating factor-mediated protein tyrosine phosphatase 1B activation by a Janus kinase 2/calpain pathway. *PLoS One* **2017,** 12, (7), e0180336.

243. Read, N. E.; Wilson, H. M., Recent Developments in the Role of Protein Tyrosine Phosphatase 1B (PTP1B) as a Regulator of Immune Cell Signalling in Health and Disease. *Int J Mol Sci* **2024,** 25, (13).

244. Braquet, P.; Rola-Pleszcynski, M., Platelet-activating factor and cellular immune responses. *Immunology Today* **1987,** 8, (11), 345-351.

245. Stafforini, D. M.; McIntyre, T. M.; Zimmerman, G. A.; Prescott, S. M., Platelet-activating factor, a pleiotrophic mediator of physiological and pathological processes. *Crit Rev Clin Lab Sci* **2003,** 40, (6), 643-72.

246. Sorrentino, R.; Terlizzi, M.; Di Crescenzo, V. G.; Popolo, A.; Pecoraro, M.; Perillo, G.; Galderisi, A.; Pinto, A., Human lung cancer-derived immunosuppressive plasmacytoid dendritic cells release IL-1α in an AIM2 inflammasome-dependent manner. *Am J Pathol* **2015,** 185, (11), 3115-24.

247. Abraham, S. N.; St. John, A. L., Mast cell-orchestrated immunity to pathogens. *Nature Reviews Immunology* **2010,** 10, (6), 440-452.

248. Plum, T.; Feyerabend, T. B.; Rodewald, H.-R., Beyond classical immunity: Mast cells as signal converters between tissues and neurons. *Immunity* **2024,** 57, (12), 2723-2736.

249. Noto, C. N.; Hoft, S. G.; DiPaolo, R. J., Mast Cells as Important Regulators in Autoimmunity and Cancer Development. *Front Cell Dev Biol* **2021,** 9, 752350.

250. Dileepan, K. N.; Raveendran, V. V.; Sharma, R.; Abraham, H.; Barua, R.; Singh, V.; Sharma, R.; Sharma, M., Mast cell-mediated immune regulation in health and disease. *Frontiers in Medicine* **2023,** Volume 10 - 2023.

251. Jones, M. K.; Nair, A.; Gupta, M., Mast Cells in Neurodegenerative Disease. *Frontiers in Cellular Neuroscience* **2019,** Volume 13 - 2019.

252. Shu, F.; Yu, J.; Liu, Y.; Wang, F.; Gou, G.; Wen, M.; Luo, C.; Lu, X.; Hu, Y.; Du, Q.; Xu, J.; Xie, R., Mast cells: key players in digestive system tumors and their interactions with immune cells. *Cell Death Discovery* **2025,** 11, (1), 8.

253. Wu, Z.; Chen, X.; Liu, F.; Chen, W.; Wu, P.; Wieschhaus, A. J.; Chishti, A. H.; Roche, P. A.; Chen, W. M.; Lin, T. J., Calpain-1 contributes to IgE-mediated mast cell activation. *J Immunol* **2014,** 192, (11), 5130-9.

254. Selvakumar, G. P.; Ahmed, M. E.; Thangavel, R.; Kempuraj, D.; Dubova, I.; Raikwar, S. P.; Zaheer, S.; Iyer, S. S.; Zaheer, A., A role for glia maturation factor dependent activation of mast cells and microglia in MPTP induced dopamine loss and behavioural deficits in mice. *Brain Behav Immun* **2020,** 87, 429-443.

255. Crocker, S. J.; Smith, P. D.; Jackson-Lewis, V.; Lamba, W. R.; Hayley, S. P.; Grimm, E.; Callaghan, S. M.; Slack, R. S.; Melloni, E.; Przedborski, S.; Robertson, G. S.; Anisman, H.; Merali, Z.; Park, D. S., Inhibition of calpains prevents neuronal and behavioral deficits in an MPTP mouse model of Parkinson's disease. *J Neurosci* **2003,** 23, (10), 4081-91.

256. Gao, A.; McCoy, H. M.; Zaman, V.; Shields, D. C.; Banik, N. L.; Haque, A., Calpain activation and progression of inflammatory cycles in Parkinson's disease. *Front Biosci (Landmark Ed)* **2022,** 27, (1), 20.

257. Kempuraj, D.; Ahmed, M. E.; Selvakumar, G. P.; Thangavel, R.; Raikwar, S. P.; Zaheer, S. A.; Iyer, S. S.; Burton, C.; James, D.; Zaheer, A., Mast Cell Activation, Neuroinflammation, and Tight Junction Protein Derangement in Acute Traumatic Brain Injury. *Mediators Inflamm* **2020,** 2020, 4243953.

258. Huang, X.; Lan, Z.; Hu, Z., Role and mechanisms of mast cells in brain disorders. *Front Immunol* **2024,** 15, 1445867.

259. Hendriksen, E.; van Bergeijk, D.; Oosting, R. S.; Redegeld, F. A., Mast cells in neuroinflammation and brain disorders. *Neurosci Biobehav Rev* **2017,** 79, 119-133.

260. Forsythe, P.; Befus, A. D., Inhibition of calpain is a component of nitric oxide-induced down-regulation of human mast cell adhesion. *J Immunol* **2003,** 170, (1), 287-93.

261. Kumar, V., Innate lymphoid cells: New paradigm in immunology of inflammation. *Immunology Letters* **2014,** 157, (1), 23-37.

262. Kumar, V., Innate lymphoid cell and adaptive immune cell cross-talk: A talk meant not to forget. *Journal of Leukocyte Biology* **2020,** 108, (1), 397-417.

263. Vivier, E.; Artis, D.; Colonna, M.; Diefenbach, A.; Di Santo, J. P.; Eberl, G.; Koyasu, S.; Locksley, R. M.; McKenzie, A. N. J.; Mebius, R. E.; Powrie, F.; Spits, H., Innate Lymphoid Cells: 10 Years On. *Cell* **2018,** 174, (5), 1054-1066.

264. Kumar, V., Innate Lymphoid Cells: Immunoregulatory Cells of Mucosal Inflammation. *European Journal of Inflammation* **2014,** 12, (1), 11-20.

265. Kumar, V., Innate Lymphoid Cells and Adaptive Immune Cells Cross-Talk: A Secret Talk Revealed in Immune Homeostasis and Different Inflammatory Conditions. *International Reviews of Immunology* **2021,** 40, (3), 217-251.

266. Diefenbach, A.; Colonna, M.; Koyasu, S., Development, Differentiation, and Diversity of Innate Lymphoid Cells. *Immunity* **2014,** 41, (3), 354-365.

267. Deshpande, R. V.; Goust, J. M.; Banik, N. L., Differential distribution of calpain in human lymphoid cells. *Neurochem Res* **1993,** 18, (7), 767-73.

268. Blom, W. M.; de Bont, H. J.; Mulder, G. J.; Nagelkerke, J. F., The role of calpains in apoptotic changes in isolated hepatocytes after attack by Natural Killer cells. *Environ Toxicol Pharmacol* **2002,** 11, (3-4), 159-65.

269. Shenoy, A. M.; Brahmi, Z., Inhibition of the calpain-mediated proteolysis of protein kinase C enhances lytic activity in human NK cells. *Cell Immunol* **1991,** 138, (1), 24-34.

270. Lee, M.; Kwon, S., Enhanced cytotoxic activity of natural killer cells from increased calcium influx induced by electrical stimulation. *PLOS ONE* **2024,** 19, (4), e0302406.

271. Prager, I.; Liesche, C.; van Ooijen, H.; Urlaub, D.; Verron, Q.; Sandström, N.; Fasbender, F.; Claus, M.; Eils, R.; Beaudouin, J.; Önfelt, B.; Watzl, C., NK cells switch from granzyme B to death receptor–mediated cytotoxicity during serial killing. *Journal of Experimental Medicine* **2019,** 216, (9), 2113-2127.

272. Ramírez-Labrada, A.; Pesini, C.; Santiago, L.; Hidalgo, S.; Calvo-Pérez, A.; Oñate, C.; Andrés-Tovar, A.; Garzón-Tituaña, M.; Uranga-Murillo, I.; Arias, M. A.; Galvez, E. M.; Pardo, J., All About (NK Cell-Mediated) Death in Two Acts and an Unexpected Encore: Initiation, Execution and Activation of Adaptive Immunity. *Front Immunol* **2022,** 13, 896228.

273. Kumar, V., Cytotoxic T Cells: Kill, Memorize, and Mask to Maintain Immune Homeostasis. *International Journal of Molecular Sciences* **2025,** 26, (18), 8788.

274. Wong, H. S.; Park, K.; Gola, A.; Baptista, A. P.; Miller, C. H.; Deep, D.; Lou, M.; Boyd, L. F.; Rudensky, A. Y.; Savage, P. A.; Altan-Bonnet, G.; Tsang, J. S.; Germain, R. N., A local regulatory T cell feedback circuit maintains immune homeostasis by pruning self-activated T cells. *Cell* **2021,** 184, (15), 3981-3997.e22.

275. Barnes, M. J.; Powrie, F., Regulatory T Cells Reinforce Intestinal Homeostasis. *Immunity* **2009,** 31, (3), 401-411.

276. Sun, L.; Su, Y.; Jiao, A.; Wang, X.; Zhang, B., T cells in health and disease. *Signal Transduction and Targeted Therapy* **2023,** 8, (1), 235.

277. Kuchay, S.; Nunez, R.; Bartholomew, A. M.; Chishti, A. H., Calpain I Null Mice Display Lymphoid Hyperplasia. *Blood* **2004,** 104, (11), 1268-1268.

278. Deshpande, R. V.; Goust, J.-M.; Chakrabarti, A. K.; Barbosa, E.; Hogan, E. L.; Banik, N. L., Calpain Expression in Lymphoid Cells: INCREASED mRNA AND PROTEIN LEVELS AFTER CELL ACTIVATION (∗). *Journal of Biological Chemistry* **1995,** 270, (6), 2497-2505.

279. Selliah, N.; Brooks, W. H.; Roszman, T. L., Proteolytic cleavage of α-actinin by calpain in T cells stimulated with anti-CD3 monoclonal antibody. *The Journal of Immunology* **1996,** 156, (9), 3215-3221.

280. Mikosik, A.; Jasiulewicz, A.; Daca, A.; Henc, I.; Frąckowiak, J. E.; Ruckemann-Dziurdzińska, K.; Foerster, J.; Le Page, A.; Bryl, E.; Fulop, T.; Witkowski, J. M., Roles of calpain-calpastatin system (CCS) in human T cell activation. *Oncotarget* **2016,** 7, (47), 76479-76495.

281. Rock, M. T.; Brooks, W. H.; Roszman, T. L., Calcium-dependent Signaling Pathways in T Cells: POTENTIAL ROLE OF CALPAIN, PROTEIN TYROSINE PHOSPHATASE 1B, AND p130Cas IN INTEGRIN-MEDIATED SIGNALING EVENTS *. *Journal of Biological Chemistry* **1997,** 272, (52), 33377-33383.

282. Wiede, F.; Lu, K. H.; Du, X.; Zeissig, M. N.; Xu, R.; Goh, P. K.; Xirouchaki, C. E.; Hogarth, S. J.; Greatorex, S.; Sek, K.; Daly, R. J.; Beavis, P. A.; Darcy, P. K.; Tonks, N. K.; Tiganis, T., PTP1B Is an Intracellular Checkpoint that Limits T-cell and CAR T-cell Antitumor Immunity. *Cancer Discov* **2022,** 12, (3), 752-773.

283. Rock, M. T.; Dix, A. R.; Brooks, W. H.; Roszman, T. L., β1 Integrin-Mediated T Cell Adhesion and Cell Spreading Are Regulated by Calpain. *Experimental Cell Research* **2000,** 261, (1), 260-270.

284. Penna, D.; Müller, S.; Martinon, F.; Demotz, S.; Iwashima, M.; Valitutti, S., Degradation of ZAP-70 following antigenic stimulation in human T lymphocytes: role of calpain proteolytic pathway. *J Immunol* **1999,** 163, (1), 50-6.

285. Damen, H.; Tebid, C.; Viens, M.; Roy, D. C.; Dave, V. P., Negative Regulation of Zap70 by Lck Forms the Mechanistic Basis of Differential Expression in CD4 and CD8 T Cells. *Front Immunol* **2022,** 13, 935367.

286. Liu, C. S. C.; Raychaudhuri, D.; Paul, B.; Chakrabarty, Y.; Ghosh, A. R.; Rahaman, O.; Talukdar, A.; Ganguly, D., Cutting Edge: Piezo1 Mechanosensors Optimize Human T Cell Activation. *The Journal of Immunology* **2018,** 200, (4), 1255-1260.

287. Liu, C. S. C.; Mandal, T.; Biswas, P.; Hoque, M. A.; Bandopadhyay, P.; Sinha, B. P.; Sarif, J.; D'Rozario, R.; Sinha, D. K.; Sinha, B.; Ganguly, D., Piezo1 mechanosensing regulates integrin-dependent chemotactic migration in human T cells. In eLife Sciences Publications, Ltd: 2024.

288. Wernimont, S. A.; Simonson, W. T. N.; Greer, P. A.; Seroogy, C. M.; Huttenlocher, A., Calpain 4 Is Not Necessary for LFA-1-Mediated Function in CD4+ T Cells. *PLOS ONE* **2010,** 5, (5), e10513.

289. Chen, J.; Ganguly, A.; Mucsi, A. D.; Meng, J.; Yan, J.; Detampel, P.; Munro, F.; Zhang, Z.; Wu, M.; Hari, A.; Stenner, M. D.; Zheng, W.; Kubes, P.; Xia, T.; Amrein, M. W.; Qi, H.; Shi, Y., Strong adhesion by regulatory T cells induces dendritic cell cytoskeletal polarization and contact-dependent lethargy. *J Exp Med* **2017,** 214, (2), 327-338.

290. Yan, J.; Liu, B.; Shi, Y.; Qi, H., Class II MHC-independent suppressive adhesion of dendritic cells by regulatory T cells in vivo. *J Exp Med* **2017,** 214, (2), 319-326.

291. Simonson, W. T. N.; Franco, S. J.; Huttenlocher, A., Talin1 Regulates TCR-Mediated LFA-1 Function1. *The Journal of Immunology* **2006,** 177, (11), 7707-7714.

292. Franco, S. J.; Rodgers, M. A.; Perrin, B. J.; Han, J.; Bennin, D. A.; Critchley, D. R.; Huttenlocher, A., Calpain-mediated proteolysis of talin regulates adhesion dynamics. *Nature Cell Biology* **2004,** 6, (10), 977-983.

293. Franz, F.; Tapia-Rojo, R.; Winograd-Katz, S.; Boujemaa-Paterski, R.; Li, W.; Unger, T.; Albeck, S.; Aponte-Santamaria, C.; Garcia-Manyes, S.; Medalia, O.; Geiger, B.; Gräter, F., Allosteric activation of vinculin by talin. *Nature Communications* **2023,** 14, (1), 4311.

294. Wang, H.; Said, R.; Nguyen-Vigouroux, C.; Henriot, V.; Gebhardt, P.; Pernier, J.; Grosse, R.; Le Clainche, C., Talin and vinculin combine their activities to trigger actin assembly. *Nature Communications* **2024,** 15, (1), 9497.

295. Mikosik, A.; Foerster, J.; Jasiulewicz, A.; Frąckowiak, J.; Colonna-Romano, G.; Bulati, M.; Buffa, S.; Martorana, A.; Caruso, C.; Bryl, E.; Witkowski, J. M., Expression of calpain-calpastatin system (CCS) member proteins in human lymphocytes of young and elderly individuals; pilot baseline data for the CALPACENT project. *Immun Ageing* **2013,** 10, (1), 27.

296. Witkowski, J. M.; Bryl, E., Paradoxical age-related cell cycle quickening of human CD4(+) lymphocytes: a role for cyclin D1 and calpain. *Exp Gerontol* **2004,** 39, (4), 577-85.

297. Perez, J.; Dansou, B.; Hervé, R.; Levi, C.; Tamouza, H.; Vandermeersch, S.; Demey-Thomas, E.; Haymann, J.-P.; Zafrani, L.; Klatzmann, D.; Boissier, M.-C.; Letavernier, E.; Baud, L., Calpains Released by T Lymphocytes Cleave TLR2 To Control IL-17 Expression. *The Journal of Immunology* **2016,** 196, (1), 168-181.

298. Frangié, C.; Zhang, W.; Perez, J.; Dubois, Y. C.; Haymann, J. P.; Baud, L., Extracellular calpains increase tubular epithelial cell mobility. Implications for kidney repair after ischemia. *J Biol Chem* **2006,** 281, (36), 26624-32.

299. Yoshimura, T.; Oppenheim, J. J., Chemerin reveals its chimeric nature. *J Exp Med* **2008,** 205, (10), 2187-90.

300. Cash, J. L.; Hart, R.; Russ, A.; Dixon, J. P.; Colledge, W. H.; Doran, J.; Hendrick, A. G.; Carlton, M. B.; Greaves, D. R., Synthetic chemerin-derived peptides suppress inflammation through ChemR23. *J Exp Med* **2008,** 205, (4), 767-75.

301. Du, X.-Y.; Leung, L. L. K., Proteolytic regulatory mechanism of chemerin bioactivity. *Acta Biochimica et Biophysica Sinica* **2009,** 41, (12), 973-979.

302. Abe, M.; Oda, N.; Sato, Y., Cell-associated activation of latent transforming growth factor-beta by calpain. *J Cell Physiol* **1998,** 174, (2), 186-93.

303. Letavernier, B.; Zafrani, L.; Nassar, D.; Perez, J.; Levi, C.; Bellocq, A.; Mesnard, L.; Sachon, E.; Haymann, J. P.; Aractingi, S.; Faussat, A. M.; Baud, L.; Letavernier, E., Calpains contribute to vascular repair in rapidly progressive form of glomerulonephritis: potential role of their externalization. *Arterioscler Thromb Vasc Biol* **2012,** 32, (2), 335-42.

304. Mikosik, A.; Zaremba, A.; Puchalska, Z.; Daca, A.; Smolenska, Z.; Lopatniuk, P.; Mital, A.; Hellman, A.; Bryl, E.; Witkowski, J. M., Ex vivo measurement of calpain activation in human peripheral blood lymphocytes by detection of immunoreactive products of calpastatin degradation. *Folia Histochem Cytobiol* **2007,** 45, (4), 343-7.

305. Mimori, T.; Suganuma, K.; Tanami, Y.; Nojima, T.; Matsumura, M.; Fujii, T.; Yoshizawa, T.; Suzuki, K.; Akizuki, M., Autoantibodies to calpastatin (an endogenous inhibitor for calcium-dependent neutral protease, calpain) in systemic rheumatic diseases. *Proceedings of the National Academy of Sciences* **1995,** 92, (16), 7267-7271.

306. Després, N.; Talbot, G.; Plouffe, B.; Boire, G.; Ménard, H. A., Detection and expression of a cDNA clone that encodes a polypeptide containing two inhibitory domains of human calpastatin and its recognition by rheumatoid arthritis sera. *The Journal of Clinical Investigation* **1995,** 95, (4), 1891-1896.

307. Iguchi-Hashimoto, M.; Usui, T.; Yoshifuji, H.; Shimizu, M.; Kobayashi, S.; Ito, Y.; Murakami, K.; Shiomi, A.; Yukawa, N.; Kawabata, D.; Nojima, T.; Ohmura, K.; Fujii, T.; Mimori, T., Overexpression of a minimal domain of calpastatin suppresses IL-6 production and Th17 development via reduced NF-κB and increased STAT5 signals. *PLoS One* **2011,** 6, (10), e27020.

308. Letavernier, E.; Dansou, B.; Lochner, M.; Perez, J.; Bellocq, A.; Lindenmeyer, M. T.; Cohen, C. D.; Haymann, J. P.; Eberl, G.; Baud, L., Critical role of the calpain/calpastatin balance in acute allograft rejection. *Eur J Immunol* **2011,** 41, (2), 473-84.

309. Smith, A. W.; Doonan, B. P.; Tyor, W. R.; Abou-Fayssal, N.; Haque, A.; Banik, N. L., Regulation of Th1/Th17 cytokines and IDO gene expression by inhibition of calpain in PBMCs from MS patients. *J Neuroimmunol* **2011,** 232, (1-2), 179-85.

310. Imam, S. A.; Guyton, M. K.; Haque, A.; Vandenbark, A.; Tyor, W. R.; Ray, S. K.; Banik, N. L., Increased calpain correlates with Th1 cytokine profile in PBMCs from MS patients. *J Neuroimmunol* **2007,** 190, (1-2), 139-45.

311. Pallotta, M. T.; Rossini, S.; Suvieri, C.; Coletti, A.; Orabona, C.; Macchiarulo, A.; Volpi, C.; Grohmann, U., Indoleamine 2,3-dioxygenase 1 (IDO1): an up-to-date overview of an eclectic immunoregulatory enzyme. *Febs j* **2022,** 289, (20), 6099-6118.

312. Forteza, M. J.; Polyzos, K. A.; Baumgartner, R.; Suur, B. E.; Mussbacher, M.; Johansson, D. K.; Hermansson, A.; Hansson, G. K.; Ketelhuth, D. F. J., Activation of the Regulatory T-Cell/Indoleamine 2,3-Dioxygenase Axis Reduces Vascular Inflammation and Atherosclerosis in Hyperlipidemic Mice. *Frontiers in Immunology* **2018,** Volume 9 - 2018.

313. Berditchevski, F.; Fennell, E.; Murray, P. G., Calcium-dependent signalling in B-cell lymphomas. *Oncogene* **2021,** 40, (45), 6321-6328.

314. Ulbricht, C.; Leben, R.; Rakhymzhan, A.; Kirchhoff, F.; Nitschke, L.; Radbruch, H.; Niesner, R. A.; Hauser, A. E., Intravital quantification reveals dynamic calcium concentration changes across B cell differentiation stages. *eLife* **2021,** 10, e56020.

315. Kitahara, A.; Ohtsuki, H.; Kirihata, Y.; Yamagata, Y.; Takano, E.; Kannagi, R.; Murachi, T., Selective localization of calpain I (the low-Ca2+-requiring form of Ca2+-dependent cysteine proteinase) in B-cells of human pancreatic islets. *FEBS Letters* **1985,** 184, (1), 120-124.

316. Ruiz-Vela, A.; Serrano, F.; González, M. A.; Abad, J. L.; Bernad, A.; Maki, M.; Martínez-A, C., Transplanted Long-Term Cultured Pre-Bi Cells Expressing Calpastatin Are Resistant to B Cell Receptor–Induced Apoptosis. *Journal of Experimental Medicine* **2001,** 194, (3), 247-254.

317. Ruiz-Vela, A.; González de Buitrago, G.; Martínez, A. C., Implication of calpain in caspase activation during B cell clonal deletion. *Embo j* **1999,** 18, (18), 4988-98.

318. Conacci-Sorrell, M.; Eisenman, R. N., Post-translational control of Myc function during differentiation. *Cell Cycle* **2011,** 10, (4), 604-10.

319. Habib, T.; Park, H.; Tsang, M.; de Alborán, I. M.; Nicks, A.; Wilson, L.; Knoepfler, P. S.; Andrews, S.; Rawlings, D. J.; Eisenman, R. N.; Iritani, B. M., Myc stimulates B lymphocyte differentiation and amplifies calcium signaling. *J Cell Biol* **2007,** 179, (4), 717-31.

320. de Barrios, O.; Meler, A.; Parra, M., MYC's Fine Line Between B Cell Development and Malignancy. *Cells* **2020,** 9, (2).

321. Calado, D. P.; Sasaki, Y.; Godinho, S. A.; Pellerin, A.; Köchert, K.; Sleckman, B. P.; de Alborán, I. M.; Janz, M.; Rodig, S.; Rajewsky, K., The cell-cycle regulator c-Myc is essential for the formation and maintenance of germinal centers. *Nat Immunol* **2012,** 13, (11), 1092-100.

322. Mikosik, A.; Henc, I.; Ruckemann-Dziurdzińska, K.; Frąckowiak, J. E.; Płoszyńska, A.; Balcerska, A.; Bryl, E.; Witkowski, J. M., Increased μ-Calpain Activity in Blasts of Common B-Precursor Childhood Acute Lymphoblastic Leukemia Correlates with Their Lower Susceptibility to Apoptosis. *PLoS One* **2015,** 10, (8), e0136615.

323. Łopatniuk, P.; Puchalska, Z.; Mital, A.; Mikosik, A.; Frąckowiak, J.; Hellmann, A.; Daca, A.; Bryl, E.; Fulop, T.; Witkowski, J. M., Excessive amount and activity of μ-calpain affects apoptotic machinery in chronic B-cell leukemia cells and influences the course of the disease. *Acta Biochim Pol* **2020,** 67, (2), 247-257.

324. Witkowski, J. M.; Zmuda-Trzebiatowska, E.; Swiercz, J. M.; Cichorek, M.; Ciepluch, H.; Lewandowski, K.; Bryl, E.; Hellmann, A., Modulation of the activity of calcium-activated neutral proteases (calpains) in chronic lymphocytic leukemia (B-CLL) cells. *Blood* **2002,** 100, (5), 1802-9.

325. Zhu, D. M.; Uckun, F. M., Calpain inhibitor II induces caspase-dependent apoptosis in human acute lymphoblastic leukemia and non-Hodgkin's lymphoma cells as well as some solid tumor cells. *Clin Cancer Res* **2000,** 6, (6), 2456-63.

326. Nothelfer, K.; Arena, E. T.; Pinaud, L.; Neunlist, M.; Mozeleski, B.; Belotserkovsky, I.; Parsot, C.; Dinadayala, P.; Burger-Kentischer, A.; Raqib, R.; Sansonetti, P. J.; Phalipon, A., B lymphocytes undergo TLR2-dependent apoptosis upon Shigella infection. *J Exp Med* **2014,** 211, (6), 1215-29.

327. Arizmendi, O.; Picking, W. D.; Picking, W. L., Macrophage Apoptosis Triggered by IpaD from Shigella flexneri. *Infect Immun* **2016,** 84, (6), 1857-1865.

328. Ono, Y.; Saido, T. C.; Sorimachi, H., Calpain research for drug discovery: challenges and potential. *Nat Rev Drug Discov* **2016,** 15, (12), 854-876.

329. Dókus, L. E.; Yousef, M.; Bánóczi, Z., Modulators of calpain activity: inhibitors and activators as potential drugs. *Expert Opin Drug Discov* **2020,** 15, (4), 471-486.

330. Leloup, L.; Wells, A., Calpains as potential anti-cancer targets. *Expert Opin Ther Targets* **2011,** 15, (3), 309-23.

331. Shapovalov, I.; Harper, D.; Greer, P. A., Calpain as a therapeutic target in cancer. *Expert Opin Ther Targets* **2022,** 26, (3), 217-231.

332. Cagmat, E. B.; Guingab-Cagmat, J. D.; Vakulenko, A. V.; Hayes, R. L.; Anagli, J., Frontiers in Neuroengineering Potential Use of Calpain Inhibitors as Brain Injury Therapy. In *Brain Neurotrauma: Molecular, Neuropsychological, and Rehabilitation Aspects*, Kobeissy, F. H., Ed. CRC Press/Taylor & Francis © 2015 by Taylor & Francis Group, LLC.: Boca Raton (FL), 2015.

333. Hu, J.; Chen, L.; Huang, X.; Wu, K.; Ding, S.; Wang, W.; Wang, B.; Smith, C.; Ren, C.; Ni, H.; ZhuGe, Q.; Yang, J., Calpain inhibitor MDL28170 improves the transplantation-mediated therapeutic effect of bone marrow-derived mesenchymal stem cells following traumatic brain injury. *Stem Cell Res Ther* **2019,** 10, (1), 96.

334. Zhang, X.; Zheng, Y.; Wang, Z.; Zhang, G.; Yang, L.; Gan, J.; Jiang, X., Calpain: The regulatory point of cardiovascular and cerebrovascular diseases. *Biomedicine & Pharmacotherapy* **2024,** 179, 117272.

335. Mader, J. S.; Mookherjee, N.; Hancock, R. E.; Bleackley, R. C., The human host defense peptide LL-37 induces apoptosis in a calpain- and apoptosis-inducing factor-dependent manner involving Bax activity. *Mol Cancer Res* **2009,** 7, (5), 689-702.

336. Barlow, P. G.; Beaumont, P. E.; Cosseau, C.; Mackellar, A.; Wilkinson, T. S.; Hancock, R. E.; Haslett, C.; Govan, J. R.; Simpson, A. J.; Davidson, D. J., The human cathelicidin LL-37 preferentially promotes apoptosis of infected airway epithelium. *Am J Respir Cell Mol Biol* **2010,** 43, (6), 692-702.

337. Duarte-Mata, D. I.; Salinas-Carmona, M. C., Antimicrobial peptides´ immune modulation role in intracellular bacterial infection. *Front Immunol* **2023,** 14, 1119574.

338. Zare-Zardini, H.; Saberian, E.; Jenča, A.; Ghanipour-Meybodi, R.; Jenča, A.; Petrášová, A.; Jenčová, J., From defense to offense: antimicrobial peptides as promising therapeutics for cancer. *Front Oncol* **2024,** 14, 1463088.

339. Teng, X.; Ji, C.; Zhong, H.; Zheng, D.; Ni, R.; Hill, D. J.; Xiong, S.; Fan, G.-C.; Greer, P. A.; Shen, Z.; Peng, T., Selective deletion of endothelial cell calpain in mice reduces diabetic cardiomyopathy by improving angiogenesis. *Diabetologia* **2019,** 62, (5), 860-872.

340. Sreenan, S. K.; Zhou, Y.-P.; Otani, K.; Hansen, P. A.; Currie, K. P. M.; Pan, C.-Y.; Lee, J.-P.; Ostrega, D. M.; Pugh, W.; Horikawa, Y.; Cox, N. J.; Hanis, C. L.; Burant, C. F.; Fox, A. P.; Bell, G. I.; Polonsky, K. S., Calpains Play a Role in Insulin Secretion and Action. *Diabetes* **2001,** 50, (9), 2013-2020.

341. Alenezi, B. T.; Elfezzani, N.; Uddin, R.; Patel, H.; Chester, S.; Abdelmaksoud, A.; Hussein, M. H.; Zaitone, S. A.; Fawzy, M. S.; Aiash, H.; Toraih, E. A., Beyond Glycemic Control: GLP-1 Receptor Agonists and Their Impact on Calcium Homeostasis in Real-World Patients. *J Clin Med* **2024,** 13, (16).

342. Kumar, V., GLP-1/GLP-1R axis: from metabolism (obesity and T2DM) to immunity. *Open Biol* **2025,** 15, (7), 240303.

343. Simpson, A. K.; Ward, P. S.; Wong, K. Y.; Collord, G. J.; Habib, A. M.; Reimann, F.; Gribble, F. M., Cyclic AMP triggers glucagon-like peptide-1 secretion from the GLUTag enteroendocrine cell line. *Diabetologia* **2007,** 50, (10), 2181-9.