immuno

MDPI

Review

Innate Immunity in Calcinosis Cutis

Simon W. Jiang, Amy J. Petty * and Matilda W. Nicholas

Department of Dermatology, Duke University School of Medicine, Durham, NC 27710, USA; simon.jiang@duke.edu (S.W.J.); matilda.nicholas@duke.edu (M.W.N.)
* Correspondence: amy.petty@duke.edu

Abstract: Calcinosis cutis is the deposition of calcium salts in the skin and subcutaneous tissue, manifesting as variably shaped papules, nodules, and plaques that can substantially impair quality of life. The pathophysiology of calcinosis cutis involves dysregulation of proinflammatory cytokines, leukocytes, and other components of the innate immune system. In some conditions associated with calcinosis cutis, elevated serum calcium, phosphate, and vitamin D may also perturb innate immunity. The mechanisms by which these lead to cutaneous and subcutaneous calcification likely parallel those seen in vascular calcification. The role of aberrant innate immunity is further supported by the association between various autoantibodies with calcinosis cutis, such as anti-MDA5, anti-NXP2, anti-centromere, and anti-topoisomerase I. Treatments for calcinosis cutis remain limited and largely experimental, although mechanistically many therapies appear to focus on dampening innate immune responses. Further research is needed to better understand the innate immune pathophysiology and establish treatment options based on randomized-controlled trials.

Keywords: calcinosis cutis; calcification; cutaneous; subcutaneous; innate immunity; innate immune system; pathophysiology; autoantibody; autoimmune; treatment; therapy

Citation: Jiang, S.W.; Petty, A.J.; Nicholas, M.W. Innate Immunity in Calcinosis Cutis. *Immuno* **2022**, *2*, 443–459. https://doi.org/10.3390/immuno2030027

Academic Editor: Young-Su Yi

Received: 11 June 2022
Accepted: 28 June 2022
Published: 1 July 2022

1. Introduction

Calcinosis cutis (CC) is the deposition and accumulation of insoluble calcium salts in the skin and subcutaneous tissue. The condition is characterized by variably shaped papules, plaques, and nodules that can cause significant pain and morbidity. CC is rare and its epidemiology is poorly understood, with few previous studies to date characterizing the incidence and prevalence of the disease. In early systemic sclerosis, CC was reported to have a prevalence of 50% [1]. Another small study by Gerami et al. reports three cases of CC in 68 patients with juvenile dermatomyositis [2].

The innate immune system plays an essential role in the skin by protecting against infection. Keratinocytes, leukocytes, antimicrobial peptides, and antiviral proteins are all important constituents of innate cutaneous immunity [3]. Dysregulation of innate immune responses play a critical role in the pathogenesis of various skin diseases, such as psoriasis, atopic dermatitis, and hidradenitis suppurativa [4–6]. However, the pathologic role of innate immunity in CC has not been previously outlined.

In this review, we aim to (1) delineate the pathogenesis of CC by whether calcification occurs due to local tissue changes or systemic abnormalities; (2) examine the roles of autoantibodies and innate immunity in the pathogenesis of CC; and (3) discuss treatments used for CC that alter innate immune processes [7]. Further studies on the mechanisms and treatments of cutaneous and subcutaneous calcification are needed to improve morbidity and quality of life in patients with CC.

2. Pathogenesis

Subtypes of CC include dystrophic calcification, idiopathic calcification, iatrogenic calcification, metastatic calcification, or calciphylaxis. Typically, earlier stages of CC involve hypoxia or trauma triggering an inflammatory response [8]. Later on, tissue degradation

and a subsequent immunological response to necrosis lead to a buildup of calcium deposits [8]. In general, investigation of the pathogenesis of cutaneous calcification is limited. Therefore, many mechanisms of tissue calcification are best understood in the context of vascular calcification. The pathogenesis of CC can be broadly divided by whether serum levels of calcium and phosphate are normal or abnormal.

2.1. Calcinosis Cutis with Normal Serum Calcium and Phosphate

CC in the setting of normocalcemia and normophosphatemia can be due to dystrophic, idiopathic, or iatrogenic calcification. Dystrophic calcification is the most common form of CC and is caused by tissue alteration or damage. Dystrophic CC is found in association with autoimmune diseases, although hereditary, neoplastic, and infectious causes are also possible [9]. Idiopathic calcification is a form of CC without tissue damage and without a well-understood cause. Three types of idiopathic CC are scrotal calcinosis, familial tumoral calcinosis, and subepidermal calcified nodules [9]. Iatrogenic calcification is due to administration of substances containing calcium or phosphate, leading to precipitation of calcium salts [10].

Cutaneous and subcutaneous calcification can be driven by proinflammatory cytokine release from macrophages. Patients with dystrophic CC and juvenile dermatomyositis (DM) have elevated levels of interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α in calcium-laden fluid collections [11,12]. These cytokines have been shown to promote osteogenic differentiation and calcification of vascular smooth muscle cells (VMSCs) in vitro [13–15]. IL-1β is produced by the NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3) inflammasome, a multiprotein oligomer assembled through the innate immune response [16]. mRNA levels of this complex were upregulated in calcifying VMSCs, and inhibition of the inflammasome via RNA interference reduced IL-1β production and VMSC calcification [17]. Furthermore, monoclonal antibody inhibition of IL-1β reduced vascular calcification in a mouse model of familial hypercholesterolemia [18]. TNF-α, a pro-inflammatory cytokine released from dendritic cells (DCs) and macrophages, plays an important role in calcifying diseases. In normophosphatemic familial tumoral calcinosis, a homozygous missense mutation results in degradation of the protein sterile α motif domain containing 9 (SAMD9), manifesting in widespread skin and mucosal calcification [19]. SAMD9 is involved in the inflammatory response to tissue injury and is regulated by TNF-α in a p38-dependent manner [19]. The role of TNF-α is further supported by a study that found elevated risk of CC in juvenile DM patients with an allele associated with increased TNF-α production [20].

IL-1β, TNF-α, and IL-6 all accelerate in vitro calcification by human adipose tissue-derived mesenchymal stem cells through increased expression of runt-related transcription factor 2 (RUNX2), a transcription factor associated with osteoblast differentiation [21]. Of these, IL-6 is the most efficacious at enhancing RUNX2 expression [21]. IL-6 increases activity of the downstream wingless-type (WNT)-5A pathway in a signal transducer and activator of transcription 3 (STAT3), but not in a STAT1-dependent manner. Interestingly, the IL-6/STAT3 pathway is activated in M2 macrophages but inhibited in M1 macrophages [22]. Although the roles of these proinflammatory cytokines have been studied in various cell types and diseases, their common involvement in tissue calcification underscores the potential importance of macrophages in the pathogenesis of dystrophic CC. Future studies should investigate the mechanisms of IL-1β, IL-6, and TNF-α specifically in CC.

Neutrophils are important players in the pathogenesis of CC [23]. One mechanism by which neutrophils contribute to host defense is the extrusion of DNA and cytoplasmic contents, forming neutrophil extracellular traps (NETs). In children with DM, tissue-infiltrating neutrophils engulfed deposits of calcium phosphate crystals, triggering cell death via generation of reactive oxygen species (ROS) and phosphoinositide 3-kinase-mediated signaling [23]. Interestingly, NET formation only occurred in proximity to calcium phosphate crystals, as NETosis was elevated in CC deposits but not plasma isolated from these juvenile DM patients [23].

Complement may also play an important role in the innate immune pathogenesis of CC. A case-control study of patients with systemic sclerosis found that the presence of CC was associated with higher serum levels of mannose-binding lectin (MBL) compared to the absence of CC [24]. The lectin complement pathway initiates when MBL (or other pattern-recognition molecules) binds PAMPs to form oligomeric complexes with MBL-associated serine protease (MASP)-1 and MASP-2 [25]. MASP-2 leads to cleavage of C4 and C2 to form C3 convertase, which triggers the complement cascade [25]. Indeed, elevated complement activity is associated with calcification in vascular diseases. In stenotic aortic valves, mRNA and protein levels of the membrane attack complex C5b-C9 and the anaphylatoxin receptor C3aR are increased [26]. In this study, C3aR expression was significantly increased by TNF-α in cultured myofibroblasts [26]. Calcifications in the abdominal aorta are also associated with elevated serum levels of MBL and the anaphylatoxins C3a and C5a [27]. One possible mechanism is multifunctional urokinase receptor (uPAR)-mediated upregulation of C5aR expression upon osteogenic differentiation of mesenchymal stem cells [28]. uPAR-knockout mice with diet-induced atherosclerosis demonstrated limited expression of C5aR and aortic calcifications compared to the control [28]. The uPAR-C5aR axis appears to mediate osteogenic differentiation through nuclear factor kappa B (NF-κB), a transcription factor that regulates many innate immune cells and pathways to promote inflammation [28]. While the precise mechanism through which complement activity contributes to CC is yet to be determined, studies of vascular calcification indicate that proinflammatory pathways are likely to be involved.

Advanced glycation end products (AGEs) are markers of oxidative stress that are found at increased levels in the dermis of systemic sclerosis patients with CC versus those without CC [29]. AGE binding to the receptor for AGEs (RAGE) initiates various signaling pathways related to cellular proliferation and inflammation, such as mitogen-activated protein kinases (MAPK)/extracellular signal-regulated kinases (ERK), stress-activated protein kinases, and janus kinase (JAK)-STAT [30]. A downstream target of RAGE activation is NF-κB, which drives transcription of proinflammatory genes through a sustained positive feedback loop [30]. Various innate immune cells in the skin express RAGE, including mononuclear phagocytes, DCs, and keratinocytes [31,32]. AGE binding to mononuclear phagocytes stimulates their chemotaxis towards skin and the production of IL-1β and TNF-α [33,34]. In DCs, exposure to AGEs induces greater production of IL-6 and activation of the nuclear factor kappa B (NF-κB) pathway [35]. Similarly, AGE stimulation of keratinocytes activates the NF-κB pathway and inhibits cellular adhesion while promoting migration [36].

AGEs may also promote calcification through hypoxic gene activation. In VSMCs, AGEs increased the expression of hypoxia-inducible factor-1α and pyruvate dehydrogenase 4, leading to vascular calcification [37]. Furthermore, metabolic pathways such as glucose metabolism, lactate production, and the oxygen consumption rate were reduced in VSMCs exposed to AGEs [37]. One study found that AGEs enhanced the production of superoxide anions and reduced the expression of superoxide dismutase in vivo. M1 macrophages are responsible for clearing ROS, although it is unclear whether an impaired response to oxidative stress in vascular calcification is indicative of macrophage polarization. Although hypoxic gene activation is an important downstream event of AGE binding to receptors in VSMCs, there are no studies that examine these pathways in CC.

2.2. Calcinosis Cutis with Abnormal Serum Calcium and Phosphate

Metastatic CC occurs when calcium precipitates in the skin in the setting of abnormal serum calcium and/or phosphate. The most common cause is chronic kidney disease (CKD), although other possible etiologies include hyperparathyroidism, hypervitaminosis D, sarcoidosis, and malignant neoplasms [10]. Calciphylaxis, or calcific uremic arteriolopathy, occurs almost exclusively in patients with CKD [38]. In this condition, arteriolar calcification in the dermis and subcutaneous fat cause ischemia and necrosis of overlying skin, leading to painful skin ulcers associated with significant morbidity [10].

Elevated serum levels of metabolically active 1,25-dihydroxyvitamin D (vitamin D_3) modulate innate immune processes in the skin. This abnormality may be seen in the setting of lymphoma, sarcoidosis, or primary hyperparathyroidism, among other conditions [39]. The receptor for vitamin D_3 (VDR) is constitutively expressed by macrophages and DCs; these cells produce vitamin D_3 via the enzyme 1α-hydroxylase and are resistant to negative feedback from 1,25-dihydroxyvitamin D [40]. Exposure to vitamin D_3 reduces the expression of major histocompatibility complex class II and costimulatory molecules (CD40, CD80, CD86) on macrophages and DCs, undermining the ability of these antigen-presenting cells to fully activate T cells [41,42]. On the other hand, vitamin D_3 enhances phagocytosis and chemotaxis by monocytes and macrophages [41]. Phagocytosis of basic calcium phosphate by macrophages in the setting of hypercalcemia activates the NLRP3 inflammasome and induces IL-1β secretion [43]. Vitamin D_3 also stimulates TNF-α production in macrophages by two possible mechanisms: increasing expression of CD14 (the receptor for lipopolysaccharide) and binding of a VDR-retinoic X receptor heterodimer to a VDR response element in the promoter region of the TNF-α gene [44].

In addition to modulating the responses of innate immune cells, vitamin D_3 may also promote calcification through antimicrobial peptides (AMPs). AMPs are peptides secreted by keratinocytes and immune cells that protect against microbial infections [45]. The AMP cathelicidin (LL-37) has both an antimicrobial function via membrane disruption and a role in mediating innate immune pathways via an alarmin-like interaction with host cells [45]. Vitamin D_3 directly stimulates cathelicidin expression via VDR response elements in the promoter region of the gene in keratinocytes and myeloid cells [46]. Cathelicidin also acts as a T cell self-antigen associated with atherosclerotic plaque calcification in T cells harvested from mice reactive to their LL-37 ortholog [47]. However, the role of cathelicidin in cutaneous and subcutaneous calcification remains unclear.

CKD is complicated by hyperphosphatemia and the accumulation of uremic toxins due to excretion failure [48]. These upregulate serum levels of proinflammatory cytokines including IL-1, IL-6, and TNF-α, which are produced by visceral adipocytes and immune cells [49]. Hyperphosphatemia induces calcification of adipocytes in vitro, possibly mediated by RUNX2 [50]. Uremic toxins promote oxidative stress through the production of ROS, leading to downstream proinflammatory responses. For instance, the uremic toxin indoxyl sulfate increases ROS production via nicotinamide adenine dinucleotide phosphate oxidase and enhances the inflammatory response and ROS generation in macrophages exposed to lipopolysaccharides [51,52]. The systemic proinflammatory environment impedes the activity of matrix Gla protein and fetuin-A (α2-Heremans-Schmid glycoprotein), proteins that inhibit soft tissue calcification [10,53,54]. Together, oxidative stress and inflammation result in significant VSMC changes, such as intimal hyperplasia, endovascular fibrosis, and differentiation into osteoblast-like cells with medial vascular calcification [55]. Subsequently, plaques, nodules, and necrotic ulcers develop due to impaired cutaneous blood supply.

2.3. Summary

CC most commonly occurs in the setting of normal serum calcium and phosphate due to dystrophic calcification from tissue damage or alteration. Pro-inflammatory cytokine release, in particular, TNF-α, IL-1, and IL-6, are involved in the pathogenesis of vascular calcification and may be relevant to the mechanisms of cutaneous and subcutaneous calcification. NET formation triggering ROS production and phosphoinositide 3-kinase-mediated signaling also contribute to calcium phosphate crystal formation in CC patients with underlying juvenile DM. Complement and AGEs are important players in vascular calcification and may play a role in CC via the activation of proinflammatory signaling pathways and hypoxic gene activation. Abnormal serum calcium and phosphate associated with CC upregulate pro-inflammatory cytokine expression and oxidative stress. This systemic inflammation impedes inhibitors of soft tissue calcification and promotes VSMC changes. Additionally, elevated serum vitamin D_3 enhances the pro-inflammatory functions

of innate immune cells. Together, these components of innate immunity play important roles in the pathogenesis of CC.

3. Autoantibody Associations

Dystrophic CC is strongly associated with underlying autoimmune disease, in particular dermatomyositis (DM) and systemic sclerosis (SSc). DM is an autoimmune disease characterized by inflammation of the muscles and the skin. CC occurs in 20–40% of patients with juvenile DM and approximately 20% of patients with adult DM [56]. Areas of the body typically affected by CC in DM include the trunk and extremities, as well as areas previously affected by inflammation and ulceration [56,57]. Scleroderma, or systemic sclerosis (SSc), is an autoimmune disorder characterized by diffuse fibrosis and inflammation of the skin and internal organs. CC occurs in approximately 20–40% of patients with SSc, depending on the population being studied [58,59]. Characteristic features of SSc, including digital ulcers and telangiectasis, are significantly associated with CC [60,61]. Other autoimmune diseases reported in association with CC include systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD), and rheumatoid arthritis [57].

3.1. Dermatomyositis

There are several autoantibodies unique to DM and other inflammatory myopathies that are relevant to the innate immune response in CC [62]. Melanoma differentiation-associated gene 5 (MDA5) is a cytosolic protein involved in host antiviral responses (Table 1). This protein senses the presence of viral RNA and induces the production of type I interferons (IFN; primarily IFN-α and IFN-β) and other proinflammatory cytokines [63]. MDA5 is strongly expressed in the epidermis of DM skin, and its presence may stimulate the chemotaxis of C-X-C motif chemokine receptor 3 (CXCR3)-expressing lymphocytes towards the dermoepidermal junction, leading to inflammation and cell death [64,65]. Subsequently, an innate immune response to released nuclear antigens may occur through toll-like receptor (TLR)-dependent and TLR-independent pathways [64]. Autoantibodies to MDA5 are associated with CC in univariate analyses of DM patients, although multivariate analyses indicate that anti-MDA5 antibodies are significantly more likely to occur in patients with fingertip ulcers [66]. The presence of this autoantibody could contribute to CC pathogenesis in DM via dysregulation of the type I IFN pathway and/or antigen-antibody binding, leading to complement fixation and antibody-dependent cytotoxicity [63].

CC is also strongly associated with autoantibodies to nuclear matrix protein 2 (NXP2) (Table 1) [56,66]. In a registry-based study examining juvenile DM patients in the United Kingdom, approximately 50% of patients with anti-NXP2 antibodies displayed CC compared to 15% of patients without these autoantibodies [74]. NXP2 is a nuclear protein that binds to RNA and regulates gene transcription by localization of promyelocytic leukemia (PML) nuclear bodies [69]. Assembly of PML nuclear bodies requires multimerization of PML, a tumor suppressor protein [75]. Notably, the PML gene contains both interferon-stimulating responsive elements (ISREs) and gamma-interferon activation sites (GAS), and its expression is therefore regulated by IFN-α, IFN-β, and IFN-γ [75]. PML induction can also occur in an IFN-independent manner; interferon regulatory factor (IRF) 3 directly binds to ISRE and GAS elements in the PML promoter without IFN synthesis [75]. The presence of anti-NXP2 antibodies may represent a PML-dependent dysregulation of host antiviral immunity in DM patients with CC.

Mitochondrial abnormalities in juvenile DM patients with CC include increased levels of cell-free mitochondrial DNA (mtDNA) in serum and the presence of anti-mitochondrial antibodies (AMA) (Table 1) [68,76]. In electron microscopy, intramitochondrial calcification was associated with degenerate muscle fibers and mitochondrial extrusion [68]. Indeed, mtDNA possesses immunogenic properties that prime innate immune responses. For instance, extracellular release of mtDNA can stimulate TLR-9 and the cyclic guanosine monophosphate–adenosine monophosphate synthase (cGAS)–stimulator of interferon genes (STING) pathway in leukocytes, activating antiviral responses via type I IFN pro-

duction [77]. Intracellular release of mtDNA from the mitochondria activates immune responses involving TLR9, cGAS, and inflammasome proteins [78]. Notably, AMA levels in juvenile DM patients correlated with the presence of the mtDNA antigen, immune complex formation, and complement C4 consumption [68]. Activation of neutrophils by mtDNA–AMA immune complexes induced the production of IL-8 and the formation of neutrophil extracellular traps, which were elevated in the peripheral blood of these patients [68]. Interestingly, AMA levels increased prior to the clinical diagnosis of CC, indicating that immunogenic responses to mtDNA release and autoantibody formation may play a role in the pathogenesis of CC [68].

Table 1. Autoantibody associations with calcinosis cutis.

Disease	Autoantibody	Role in Innate Immunity	References
All	Anti-nuclear	[a] Forms immune complexes with cognate antigens, inducing the production of type I IFNs and other cytokines	[67]
Dermatomyositis	Anti-MDA5	[b] Senses viral RNA and induces type I IFN production [b] Stimulates chemotaxis of CXCR3+ lymphocytes to the dermoepidermal junction	[63–65]
	Anti-mitochondrial	[a] Forms immune complexes with mtDNA, inducing IL-8 production and NET formation	[68]
	Anti-NXP2	[b] Binds RNA and regulates transcription by localization of PML nuclear bodies	[69]
MCTD	Anti-U1-RNP	[a] Forms immune complexes with cognate antigens, inducing the production of proinflammatory cytokines	[70]
Systemic sclerosis	Anti-centromere (centromere-kinetochore macrocomplex)	[b] Associates with nuclear cGAS, activating STING and stimulating NF-κB and IRF3 expression	[71]
	Anti-DNA topoisomerase I	[b] Activates the transcription of proinflammatory genes via positive regulation of RNA polymerase II [b] Triggers antiviral immunity through cGAS-STING signaling *	[72,73]

Abbreviations: cGAS = cyclic guanosine monophosphate-adenosine monophosphate synthase; CXCR3 = C-X-C Motif Chemokine Receptor 3; IFN = interferon; IL = interleukin; IRF = interferon regulatory factor; MCTD = mixed connective tissue disease; MDA5 = melanoma differentiation-associated gene 5; NET = neutrophil extracellular trap; NF-κB = nuclear factor kappa B; NXP2 = nuclear matrix protein 2; PML = promyelocytic leukemia; STING = stimulator of interferon genes; U1 RNP = U1 small nuclear ribonucleoprotein. * As a result of minor DNA damage from its inhibition [a] Role of autoantibody [b] Role of antigen.

3.2. Systemic Sclerosis

Anti-centromere antibodies (ACA) are associated with CREST (calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia) syndrome, the limited cutaneous form of SSc [79]. A cross-sectional study of SSc patients found that CC is associated with ACAs in a multivariate analysis [60]. ACAs target components of the centromere–kinetochore macrocomplex, which binds sister chromatids together and serves as an attachment for spindle fibers (Table 1) [80]. Nuclear cGAS preferentially associates with centromeres through its N-terminal domain [71]. Interestingly, the foci on centromeres to which cGAS binds are the same foci to which ACAs bind [71]. cGAS senses cytosolic DNA and activates STING, which stimulates NF-κB and IRF3 expression [71]. Subsequently, a type I IFN response with IFN-stimulated gene expression and DC activation occurs [71]. The association between ACAs and CC could represent cGAS-mediated upregulation of type I IFN responses.

Autoantibodies to DNA topoisomerase I are more frequently found in SSc patients with CC than in those without CC (Table 1) [58]. Topoisomerase I enzymes are primarily responsible for relaxing supercoiled double-stranded DNA during replication. However,

the regulation of topoisomerase I activity can also modulate host innate immune responses. In response to host infection, topoisomerase I activates the transcription of proinflammatory genes via the positive regulation of RNA polymerase II [72]. Inhibition of topoisomerase I rescues lethal inflammation caused by viral and bacterial pathogen-associated molecular patterns (PAMPs) in mouse models [72]. On the other hand, topoisomerase I inhibition causing minor DNA damage can also trigger an antiviral response through the cGAS-STING pathway via detection of cytosolic DNA [73]. It is unclear whether autoantibodies to DNA topoisomerase I in CC signify a heightened cGAS-mediated immune response or if they represent an attempt to dampen inflammatory processes.

3.3. Other Autoimmune Diseases

Elevated titers of antinuclear antibodies (ANA) are found in numerous autoimmune diseases, such as SLE, DM, and SSc. While no large-scale retrospective study has identified a clear association between ANA and CC, numerous case reports and series have found a potential link. A case series of patients with chronic graft-versus-host disease found that four out of five patients with CC had elevated ANA titers [81]. Diffuse CC has been described in patients with Sjogren's syndrome and SLE with positive ANA [82,83]. Charles and colleagues found that basophils were activated by autoreactive IgE home to secondary lymphoid tissue, promoting the differentiation of T helper type 2 cells. This results in the production of autoantibodies including ANA in mice that lack the Src family protein kinase Lyn [84]. ANAs can subsequently form immune complexes with their cognate antigens, interacting with nucleic acid sensors in host immune cells to induce the production of type I IFNs and other cytokines (Table 1) [67].

MCTD is a rare inflammatory autoimmune disease with clinical features of SLE, SSc, and polymyositis. MCTD is characterized by autoantibodies to the U1 small nuclear ribonucleoprotein antigen (U1-RNP) and has been shown to be associated with CC in a limited number of patients (Table 1) [57,85–87]. U1-RNP is conventionally involved in alternative splicing following synthesis of mRNA transcripts that contain introns and exons. Similar to ANAs, anti-U1-RNP antibodies can form immune complexes with self RNA, serving as ligands for nucleic acid sensors that activate downstream immune responses such as the production of proinflammatory cytokines and the activation of DCs and lymphocytes [70]. TLR3 in particular is important in MCTD pathogenesis, as TLR3-deficient mice treated with U1-RNP and U1-RNA developed an autoimmune syndrome typical of SLE but lacking the interstitial lung disease of MCTD [88]. Perhaps this receptor is similarly important for the development of CC lesions, although in vivo studies of this potential role are lacking.

3.4. Summary

Dystrophic CC is strongly associated with underlying autoimmune disease, especially DM and SSc. In DM, the presence of autoantibodies such as anti-MDA5 and anti-NXP2 are likely indicative of dysregulated antiviral pathways. AMAs may also complex with mtDNA, activating neutrophils and complement. An altered antiviral response is further supported by the presence of ACAs and anti-topoisomerase I antibodies in SSc. Finally, ANAs and anti-U1 RNP antibodies may contribute to excess activation of pro-inflammatory pathways and cytokine production via the formation of immune complexes with self-antigens. While many other autoantibodies are associated with CC, the aforementioned are most indicative of a dysregulated innate immune response.

4. Treatment

Therapies for CC are largely experimental. Most evidence of treatment efficacy derives from case reports and series, although there are several retrospective studies with larger sample sizes. Prospective and/or randomized controlled trials are limited. Currently, there are no therapies for CC approved by the US Food and Drug Administration.

4.1. Bisphosphonates

Bisphosphonates are synthetic analogs of inorganic pyrophosphate, a byproduct of many reactions that occur in the body [89]. Their primary use is in disorders of bone loss mediated by osteoclasts, such as osteoporosis, Paget disease of bone, and primary or metastatic bone tumors [89]. In these diseases, bisphosphonates bind to hydroxyapatite crystals and are preferentially incorporated into areas of active bone remodeling, inhibiting calcification and bone resorption [89].

The utility of bisphosphonates for treatment of CC has been substantiated by several case series that demonstrated partial or complete response in a majority of patients studied [8,90,91]. Another study found that the bisphosphonate etidronate was unsuccessful in treatment of CC with underlying DM or SSc [92]. Though their mechanism of action in CC remains unclear, one possible explanation is via reduced systemic calcium, which limits calcification of the skin and subcutaneous tissue. However, as discussed earlier, a majority of CC cases occur due to local tissue abnormalities in the setting of normocalcemia. In these cases, bisphosphonates may function through the inhibition of monocytes and macrophages (Table 2). Exposure to unbound bisphosphonate reduces macrophage viability and cell number and induces apoptosis [93]. Macrophages are highly endocytic and can uptake unbound bisphosphonate circulating in the blood and/or released from bone via fluid-phase endocytosis [94]. In animal models of solid tumors such as lung, breast, and liver cancer, bisphosphonates reduce the infiltration of tumor-associated macrophages into the tumoral stroma and may even reverse their polarity from pro-tumoral M2 macrophages to tumoricidal M1 macrophages [95]. Therefore, it is possible that bisphosphonates similarly reduce macrophage viability, proliferation, and infiltration within the microenvironment of dystrophic CC lesions.

Table 2. Treatments for calcinosis cutis associated with the modulation of innate immunity.

Treatment	Subclass	Mechanism	References
Bisphosphonates	-	Reduces monocyte/macrophage cell number and viability and induces apoptosis	[93]
Antibiotics	Minocycline	Reduces TNF-α, IL-1, and IL-6, inhibits neutrophil chemotaxis, and suppresses of MMP activity	[96–98]
	Ceftriaxone	Reduces TNF-α and suppresses MMP activity	[98]
Colchicine	-	Inhibits neutrophil chemotaxis and NETosis, suppresses NLRP3 inflammasome activation, and reduces proinflammatory cytokine release by macrophages	[99,100]
Corticosteroids	-	Reduces pro-inflammatory cytokine release, decreases circulating innate immune cells, and suppresses fibroblast growth and TGF-β1 production Reduces vessel permeability	[101,102]
IVIG	-	Suppresses the production of pro-inflammatory cytokines in CD16+ intermediate monocytes Inhibits DC maturation and differentiation, reducing IL-12 secretion and the expression of costimulatory molecules	[103,104]
Biologics	Adalimumab	Inhibits TNF-α	[105]
	Infliximab	Inhibits TNF-α	[106]

Abbreviations: DC = dendritic cell; IL = interleukin; IVIG = intravenous immunoglobulin; MMP = matrix metalloprotease; NET = neutrophil extracellular trap; TGF-β1 = transforming growth factor beta 1; TNF-α = tumor necrosis factor alpha.

4.2. Antibiotics

In addition to their bacteriostatic and bactericidal properties, antibiotics can dampen inflammatory processes, which may account for their role in the treatment of CC. The antibiotic minocycline has demonstrated efficacy in reducing ulceration and the size of CC

deposits in a prospective trial of 8/9 patients with limited cutaneous SSc [107]. In a study of five dogs with CC, four achieved complete remission with a reduction in the size of calcinosis deposits and associated inflammation [108]. Similarly, the cephalosporin ceftriaxone has been used in the treatment of CC, although its evidence is limited. In an adolescent male with subcutaneous morphea associated with dystrophic calcification, ceftriaxone demonstrated a favorable response after 20 days of intravenous administration [109].

The anti-inflammatory properties of minocycline include the inhibition of TNF-α, IL-1, and IL-6, as well as reduction of neutrophil chemotaxis [96,97]. Ceftriaxone transiently reduces serum levels of TNF-α in vivo [110]. Minocycline and ceftriaxone may also reduce calcinosis deposits via the inhibition of matrix metalloproteinase (MMP) activity [98]. MMPs are enteropeptidases produced by activated fibroblasts that induce tissue destruction via the degradation of the extracellular matrix [111]. They are pivotal players in the innate immune response via the modulation of chemokine activity and the establishment of chemokine gradients, controlling the influx of neutrophils, eosinophils, and monocytes [112]. In rat models with CKD, VSMCs surrounding areas of medial calcification had increased expression of MMP-2 and MMP-9 [113]. Hyperphosphatemia increased the expression of MMPs in VSMCs from normal rats but not CKD rats, while the inhibition of MMP activity reduced vascular calcification in both normal rats and CKD rats. Therefore, antibiotics such as minocycline and ceftriaxone may diminish cutaneous and subcutaneous calcification via the modulation of pro-inflammatory processes and MMP activity.

4.3. Colchicine

Colchicine is a plant alkaloid extracted from Colchicum that binds to tubulin to prevent microtubule assembly and polymerization. This leads to a widespread modulation of innate immune activity, such as the inhibition of neutrophil chemotaxis and adhesion, the suppression of NLRP3 inflammasome activation, and the reduction of proinflammatory cytokine production by macrophages [99]. The drug is most commonly used in gouty arthritis and Familial Mediterranean Fever, although its utility has expanded to other diseases such as pericarditis and atherosclerosis [99].

There is limited evidence of the efficacy of colchicine in CC therapy. One study identified a partial response in 3/7 patients, while another study found a partial response in only 1/9 patients [57,114]. Fuchs and colleagues observed that colchicine improved local inflammation and ulcer healing in two patients but did not impact the degree of calcification in their lesions [100]. The heterogeneity of responses in patients with CC to this medication may be related to the various mechanisms by which it exerts immunological effects. Colchicine reduced NETosis from neutrophils in animal models of acute respiratory distress syndrome, which was associated with a reduction in acute lung injury [115]. Similarly, NETosis was reduced in patients with acute coronary syndrome treated with colchicine after percutaneous coronary intervention [116]. Perhaps NETosis plays a more dominant role in the pathogenesis of colchicine-responsive CC, especially in those who experience a reduction in calcification.

4.4. Corticosteroids and Intravenous Immunoglobulin

Corticosteroids have a broad and diverse mechanism of anti-inflammatory action, including the inhibition of pro-inflammatory cytokine release, a decrease in circulating innate immune cells, and reduced vessel permeability [101]. Intralesional injections of corticosteroids have demonstrated limited efficacy in the treatment of CC deposits. Case reports from 1978 and 1982 described an improvement of CC deposits treated with intralesional steroids in an adolescent male with calcinosis cutis circumscripta and an elderly female with scleroderma, respectively [117,118]. In a patient with juvenile DM refractory to intravenous bisphosphonate and colchicine, intralesional steroid injection resulted in complete resolution of a CC deposit in the left elbow without relapse at two-year follow-up [119]. Regarding their intralesional application, corticosteroids inhibit fibroblast growth and transforming growth factor β1, a cytokine with many roles in cell growth, differentiation,

and apoptosis [102]. The regulation of fibroblast activity also reduces MMP secretion, which may further inhibit cutaneous calcification.

Polyvalent intravenous immunoglobulins (IVIG) are directed against a broad spectrum of microbial and self-antigens. IVIG can be used for the treatment of autoimmune disorders and can secondarily impact dystrophic CC. Case reports of patients receiving IVIG therapy for underlying DM or CREST syndrome have demonstrated efficacy against dystrophic CC lesions [120,121]. However, the treatment response appears to be variable; one group found that a DM patient receiving IVIG failed to experience a reduction in dystrophic CC, while another found that 3/8 patients demonstrated stable or worsened CC after receiving IVIG therapy [122,123]. At the high dosages used to treat rheumatologic disorders, IVIG primarily exerts anti-inflammatory properties to modulate innate immunity. Although in vivo studies indicate that IVIG does not significantly impact neutrophil activity, in vitro data demonstrate that IVIG promotes apoptosis and inhibits degranulation in neutrophils [103]. On the other hand, IVIG transiently suppresses the production of pro-inflammatory cytokines in CD16+ pro-inflammatory intermediate monocytes [103]. This may occur via apoptosis, as IVIG contains anti-CD95 antibodies that interact with CD16+ monocytes expressing constitutively upregulated genes promoting apoptosis [103,124]. High-dose IVIG also inhibits DC maturation and differentiation, reducing IL-12 secretion and the expression of costimulatory molecules [104]. Taken together, IVIG likely reduces CC deposits in some patients by suppressing proinflammatory pathways that involve innate immunity.

4.5. Biologics

Monoclonal antibody therapy directed against specified antigen targets has the potential to not only elicit a more tailored clinical response but also to elucidate the role of the targets in CC pathogenesis. TNF-α inhibitors such as adalimumab and infliximab are widely used for the treatment of autoimmune disorders and may secondarily treat CC lesions given the role of this cytokine in disease mechanisms. In a case report of a DM patient with extensive calcinosis refractory to corticosteroids, IVIG, and rituximab, treatment with adalimumab resulted in an improvement in both CC and DM with maintained remission three years after discontinuing therapy [105]. Interestingly, the patient received etanercept instead of adalimumab during pregnancy, and her treatment response was maintained [105]. Another case report of a patient with SSc/myositis overlap syndrome and recalcitrant calcinosis observed a reduction in the size of CC lesions with no new lesions at 41 months of infliximab therapy [106].

Rituximab is a chimeric monoclonal antibody directed against CD20, a receptor primarily found on B cells. Despite the role of these cells in adaptive immunity, rituximab therapy for CC will be briefly discussed given the relative wealth of literature available. In a case series of six patients with CC and SSc, rituximab resulted in an improvement of calcinosis lesions in three patients [125]. Another case series found complete resolution of CC lesions in three patients with SSc within six months of rituximab therapy, without relapse at follow-up [126]. However, in a prospective study of juvenile DM patients, CC deposits did not improve in the six affected patients after rituximab therapy [127]. A randomized controlled trial examined the efficacy of rituximab therapy in seven patients with adult DM and 22 patients with juvenile DM and concomitant CC [128]. Since partial response was not assessed, Aggarwal and colleagues found that only 1/7 adult DM and 1/22 juvenile DM patients experienced complete resolution of their CC deposits [128].

The heterogeneous response to different therapies, especially biologics, in CC suggests that different disease phenotypes may exist. Phenotypic classification based on genetic susceptibility or serum markers may guide therapy as in other skin diseases. For instance, in psoriasis, ustekinumab therapy for CXCR3+ CCR6− CD38+ HLA-DR+ activated Th1 cell-predominant disease and secukinumab therapy for CXCR3− CCR6+ CD38+ HLA-DR+ Th17 cell-predominant disease resulted in a significantly lower psoriasis area and severity index at six months of strategic treatment [129]. Furthermore, treatment of underlying rheumatologic disease may alter the innate and adaptive immunophenotype and impact

the response of CC lesions to subsequent therapy. In juvenile DM, naïve B and CD4+ T cells were found at increased serum levels while natural killer (NK) cells were found at decreased serum levels compared to controls in treatment-naïve patients [130]. NK cells in particular demonstrated hypophosphorylation of phospholipase Cγ2, an enzyme that stimulates calcium flux and the movement of cytotoxic granules [130]. Treatment of active disease attenuated cell frequency differences in naïve B cells, CD4+ T cells, and NK cells, as well as hypophosphorylation of phospholipase Cγ2 and downstream MAPK2 signaling [130]. Therefore, it is possible that CC lesions have varied immunophenotypes requiring different targeted therapies, which may be impacted by concomitant treatment for underlying autoimmune diseases.

4.6. Summary

While treatment for CC is limited by the lack of large, randomized-controlled studies, the existing evidence suggests that the available therapy modulates innate immune responses. Bisphosphonates inhibit monocyte/macrophage function and may alter macrophage polarity in dystrophic CC lesions. Antibiotics have broad anti-inflammatory action and suppress MMP activity, potentially reducing cutaneous and subcutaneous calcification. Neutrophil activation and NETosis in CC are targeted by colchicine, although response to therapy is variable. The broad anti-inflammatory actions of corticosteroids and IVIG may limit CC development through the inhibition of fibroblast activity and cytokine production from pro-inflammatory CD16+ monocytes, respectively. Finally, biologics such as adalimumab, infliximab, and rituximab target specific components of innate and adaptive immunity. The next steps towards improving the treatment of CC include a better understanding of heterogenous responses towards therapy and the investigation of possible immunophenotypes.

5. Conclusions

Calcinosis cutis is the deposition of insoluble calcium salts in the skin and subcutaneous tissue. Although the disease is poorly understood, the innate immune system plays an important role in its pathophysiology and treatment. Elevated serum and lesion levels of TNF-α, IL-1, IL-6, NETs, complement, and AGEs demonstrate the important role of innate immunity in patients with CC and normal calcium and phosphate levels. These pathways are dysregulated in vascular calcification and may be similarly perturbed in CC. In patients with elevated calcium and/or phosphate levels, CC pathophysiology is also related to pro-inflammatory cytokine dysregulation contributing to ROS production and soft tissue calcification. CC is strongly associated with autoimmune diseases, in particular dermatomyositis and systemic sclerosis. Autoantibody associations in these rheumatologic disorders with CC such as anti-MDA5, anti-NXP2, anti-centromere, and anti-topoisomerase I further support the role of aberrant antiviral and pro-inflammatory cytokine activation in CC pathophysiology. The data supporting medical therapy for CC are limited and mostly experimental, as there are currently no FDA-approved treatments. However, the available literature supports the use of bisphosphonates, antibiotics, colchicine, corticosteroids, IVIG, and biologics, therapies that likely impact CC deposits via the modulation of innate immune responses. Further studies are needed to better understand the pathophysiology and treatment of calcinosis cutis in order to improve the quality of life and morbidity of patients afflicted with this disease.

Author Contributions: Conceptualization, S.W.J., A.J.P. and M.W.N.; Writing—original draft preparation, S.W.J.; Writing—reviewing & editing, A.J.P. and M.W.N. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: This study did not report any data.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Muktabhant, C.; Thammaroj, P.; Chowchuen, P.; Foocharoen, C. Prevalence and Clinical Association with Calcinosis Cutis in Early Systemic Sclerosis. *Mod. Rheumatol.* **2021**, *31*, 1113–1119. [CrossRef] [PubMed]
2. Gerami, P.; Walling, H.W.; Lewis, J.; Doughty, L.; Sontheimer, R.D. A Systematic Review of Juvenile-Onset Clinically Amyopathic Dermatomyositis. *Br. J. Dermatol.* **2007**, *157*, 637–644. [CrossRef] [PubMed]
3. Coates, M.; Blanchard, S.; MacLeod, A.S. Innate Antimicrobial Immunity in the Skin: A Protective Barrier against Bacteria, Viruses, and Fungi. *PLoS Pathog.* **2018**, *14*, e1007353. [CrossRef] [PubMed]
4. Lanna, C.; Mancini, M.; Gaziano, R.; Cannizzaro, M.V.; Galluzzo, M.; Talamonti, M.; Rovella, V.; Annicchiarico-Petruzzelli, M.; Melino, G.; Wang, Y.; et al. Skin Immunity and Its Dysregulation in Psoriasis. *Cell Cycle* **2019**, *18*, 2581–2589. [CrossRef] [PubMed]
5. Yang, G.; Seok, J.K.; Kang, H.C.; Cho, Y.-Y.; Lee, H.S.; Lee, J.Y. Skin Barrier Abnormalities and Immune Dysfunction in Atopic Dermatitis. *Int. J. Mol. Sci.* **2020**, *21*, 2867. [CrossRef]
6. Jiang, S.W.; Whitley, M.J.; Mariottoni, P.; Jaleel, T.; MacLeod, A.S. Hidradenitis Suppurativa: Host-Microbe and Immune Pathogenesis Underlie Important Future Directions. *JID Innov.* **2021**, *1*, 100001. [CrossRef]
7. Róbert, L.; Kiss, N.; Medvecz, M.; Kuroli, E.; Sárdy, M.; Hidvégi, B. Epidemiology and Treatment of Calcinosis Cutis: 13 Years of Experience. *Indian J. Dermatol.* **2020**, *65*, 105–111. [CrossRef]
8. Rauch, L.; Hein, R.; Biedermann, T.; Eyerich, K.; Lauffer, F. Bisphosphonates for the Treatment of Calcinosis Cutis-A Retrospective Single-Center Study. *Biomedicines* **2021**, *9*, 1698. [CrossRef]
9. Reiter, N.; El-Shabrawi, L.; Leinweber, B.; Berghold, A.; Aberer, E. Calcinosis Cutis: Part I. Diagnostic Pathway. *J. Am. Acad. Dermatol.* **2011**, *65*, 1–12. [CrossRef]
10. Jiménez-Gallo, D.; Ossorio-García, L.; Linares-Barrios, M. Calcinosis Cutis and Calciphylaxis. *Actas Dermosifiliogr.* **2015**, *106*, 785–794. [CrossRef]
11. Mukamel, M.; Horev, G.; Mimouni, M. New Insight into Calcinosis of Juvenile Dermatomyositis: A Study of Composition and Treatment. *J. Pediatr.* **2001**, *138*, 763–766. [CrossRef] [PubMed]
12. Shimizu, M.; Ueno, K.; Ishikawa, S.; Kasahara, Y.; Yachie, A. Role of Activated Macrophage and Inflammatory Cytokines in the Development of Calcinosis in Juvenile Dermatomyositis. *Rheumatology* **2014**, *53*, 766–767. [CrossRef] [PubMed]
13. Ikeda, K.; Souma, Y.; Akakabe, Y.; Kitamura, Y.; Matsuo, K.; Shimoda, Y.; Ueyama, T.; Matoba, S.; Yamada, H.; Okigaki, M.; et al. Macrophages Play a Unique Role in the Plaque Calcification by Enhancing the Osteogenic Signals Exerted by Vascular Smooth Muscle Cells. *Biochem. Biophys. Res. Commun.* **2012**, *425*, 39–44. [CrossRef] [PubMed]
14. Lencel, P.; Delplace, S.; Pilet, P.; Leterme, D.; Miellot, F.; Sourice, S.; Caudrillier, A.; Hardouin, P.; Guicheux, J.; Magne, D. Cell-Specific Effects of TNF-α and IL-1β on Alkaline Phosphatase: Implication for Syndesmophyte Formation and Vascular Calcification. *Lab. Investig.* **2011**, *91*, 1434–1442. [CrossRef]
15. Kurozumi, A.; Nakano, K.; Yamagata, K.; Okada, Y.; Nakayamada, S.; Tanaka, Y. IL-6 and SIL-6R Induces STAT3-Dependent Differentiation of Human VSMCs into Osteoblast-like Cells through JMJD2B-Mediated Histone Demethylation of RUNX2. *Bone* **2019**, *124*, 53–61. [CrossRef]
16. Lopez-Castejon, G.; Brough, D. Understanding the Mechanism of IL-1β Secretion. *Cytokine Growth Factor Rev.* **2011**, *22*, 189–195. [CrossRef]
17. Wen, C.; Yang, X.; Yan, Z.; Zhao, M.; Yue, X.; Cheng, X.; Zheng, Z.; Guan, K.; Dou, J.; Xu, T.; et al. Nalp3 Inflammasome Is Activated and Required for Vascular Smooth Muscle Cell Calcification. *Int. J. Cardiol.* **2013**, *168*, 2242–2247. [CrossRef]
18. Awan, Z.; Denis, M.; Roubtsova, A.; Essalmani, R.; Marcinkiewicz, J.; Awan, A.; Gram, H.; Seidah, N.G.; Genest, J. Reducing Vascular Calcification by Anti-IL-1β Monoclonal Antibody in a Mouse Model of Familial Hypercholesterolemia. *Angiology* **2016**, *67*, 157–167. [CrossRef]
19. Chefetz, I.; Ben Amitai, D.; Browning, S.; Skorecki, K.; Adir, N.; Thomas, M.G.; Kogleck, L.; Topaz, O.; Indelman, M.; Uitto, J.; et al. Normophosphatemic Familial Tumoral Calcinosis Is Caused by Deleterious Mutations in SAMD9, Encoding a TNF-Alpha Responsive Protein. *J. Investig. Dermatol.* **2008**, *128*, 1423–1429. [CrossRef]
20. Pachman, L.M.; Liotta-Davis, M.R.; Hong, D.K.; Kinsella, T.R.; Mendez, E.P.; Kinder, J.M.; Chen, E.H. TNFalpha-308A Allele in Juvenile Dermatomyositis: Association with Increased Production of Tumor Necrosis Factor Alpha, Disease Duration, and Pathologic Calcifications. *Arthritis Rheum.* **2000**, *43*, 2368–2377. [CrossRef]
21. Fukuyo, S.; Yamaoka, K.; Sonomoto, K.; Oshita, K.; Okada, Y.; Saito, K.; Yoshida, Y.; Kanazawa, T.; Minami, Y.; Tanaka, Y. IL-6-Accelerated Calcification by Induction of ROR2 in Human Adipose Tissue-Derived Mesenchymal Stem Cells Is STAT3 Dependent. *Rheumatology* **2014**, *53*, 1282–1290. [CrossRef] [PubMed]
22. Yin, Z.; Ma, T.; Lin, Y.; Lu, X.; Zhang, C.; Chen, S.; Jian, Z. IL-6/STAT3 Pathway Intermediates M1/M2 Macrophage Polarization during the Development of Hepatocellular Carcinoma. *J. Cell. Biochem.* **2018**, *119*, 9419–9432. [CrossRef] [PubMed]
23. Duvvuri, B.; Pachman, L.M.; Morgan, G.; Khojah, A.M.; Klein-Gitelman, M.; Curran, M.L.; Doty, S.; Lood, C. Neutrophil Extracellular Traps in Tissue and Periphery in Juvenile Dermatomyositis. *Arthritis Rheumatol.* **2020**, *72*, 348–358. [CrossRef]

24. Osthoff, M.; Ngian, G.-S.; Dean, M.M.; Nikpour, M.; Stevens, W.; Proudman, S.; Eisen, D.P.; Sahhar, J. Potential Role of the Lectin Pathway of Complement in the Pathogenesis and Disease Manifestations of Systemic Sclerosis: A Case-Control and Cohort Study. *Arthritis Res. Ther.* **2014**, *16*, 480. [CrossRef]
25. Dávid, H.; Andrea, K.; József, D.; Katalin, S.; Róbert, S.; Péter, Z.; Gábor, P.; Péter, G. Revised Mechanism of Complement Lectin-Pathway Activation Revealing the Role of Serine Protease MASP-1 as the Exclusive Activator of MASP-2. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 10498–10503. [CrossRef]
26. Helske, S.; Oksjoki, R.; Lindstedt, K.A.; Lommi, J.; Turto, H.; Werkkala, K.; Kupari, M.; Kovanen, P.T. Complement System Is Activated in Stenotic Aortic Valves. *Atherosclerosis* **2008**, *196*, 190–200. [CrossRef] [PubMed]
27. Wang, Y.; Miao, Y.; Gong, K.; Cheng, X.; Chen, Y.; Zhao, M.-H. Plasma Complement Protein C3a Level Was Associated with Abdominal Aortic Calcification in Patients on Hemodialysis. *J. Cardiovasc. Transl. Res.* **2019**, *12*, 496–505. [CrossRef]
28. Kalbasi Anaraki, P.; Patecki, M.; Larmann, J.; Tkachuk, S.; Jurk, K.; Haller, H.; Theilmeier, G.; Dumler, I. Urokinase Receptor Mediates Osteogenic Differentiation of Mesenchymal Stem Cells and Vascular Calcification via the Complement C5a Receptor. *Stem Cells Dev.* **2014**, *23*, 352–362. [CrossRef]
29. Davies, C.A.; Herrick, A.L.; Cordingley, L.; Freemont, A.J.; Jeziorska, M. Expression of Advanced Glycation End Products and Their Receptor in Skin from Patients with Systemic Sclerosis with and without Calcinosis. *Rheumatology* **2009**, *48*, 876–882. [CrossRef]
30. Gkogkolou, P.; Böhm, M. Advanced Glycation End Products: Key Players in Skin Aging? *Dermato-Endocrinol.* **2012**, *4*, 259–270. [CrossRef]
31. Lohwasser, C.; Neureiter, D.; Weigle, B.; Kirchner, T.; Schuppan, D. The Receptor for Advanced Glycation End Products Is Highly Expressed in the Skin and Upregulated by Advanced Glycation End Products and Tumor Necrosis Factor-Alpha. *J. Investig. Dermatol.* **2006**, *126*, 291–299. [CrossRef] [PubMed]
32. Fujimoto, E.; Kobayashi, T.; Fujimoto, N.; Akiyama, M.; Tajima, S.; Nagai, R. AGE-Modified Collagens I and III Induce Keratinocyte Terminal Differentiation through AGE Receptor CD36: Epidermal-Dermal Interaction in Acquired Perforating Dermatosis. *J. Investig. Dermatol.* **2010**, *130*, 405–414. [CrossRef]
33. Schmidt, A.M.; Yan, S.D.; Brett, J.; Mora, R.; Nowygrod, R.; Stern, D. Regulation of Human Mononuclear Phagocyte Migration by Cell Surface-Binding Proteins for Advanced Glycation End Products. *J. Clin. Investig.* **1993**, *91*, 2155–2168. [CrossRef] [PubMed]
34. Jin, X.; Liu, L.; Zhang, Y.; Xiang, Y.; Yin, G.; Lu, Y.; Shi, L.; Dong, J.; Shen, C. Advanced Glycation End Products Enhance Murine Monocyte Proliferation in Bone Marrow and Prime Them into an Inflammatory Phenotype through MAPK Signaling. *J. Diabetes Res.* **2018**, *2018*, 2527406. [CrossRef] [PubMed]
35. Hilmenyuk, T.; Bellinghausen, I.; Heydenreich, B.; Ilchmann, A.; Toda, M.; Grabbe, S.; Saloga, J. Effects of Glycation of the Model Food Allergen Ovalbumin on Antigen Uptake and Presentation by Human Dendritic Cells. *Immunology* **2010**, *129*, 437–445. [CrossRef] [PubMed]
36. Tian, M.; Lu, S.; Niu, Y.; Xie, T.; Dong, J.; Cao, X.; Song, F. Effects of Advanced Glycation End-Products (AGEs) on Skin Keratinocytes by Nuclear Factor-Kappa B (NF-KB) Activation. *Afr. J. Biotechnol.* **2012**, *11*, 11132–11142. [CrossRef]
37. Zhu, Y.; Ma, W.-Q.; Han, X.-Q.; Wang, Y.; Wang, X.; Liu, N.-F. Advanced Glycation End Products Accelerate Calcification in VSMCs through HIF-1α/PDK4 Activation and Suppress Glucose Metabolism. *Sci. Rep.* **2018**, *8*, 13730. [CrossRef]
38. Altman, K.; Shinohara, M. Demographics, Comorbid Conditions, and Outcomes of Patients with Nonuremic Calciphylaxis. *JAMA Dermatol.* **2019**, *155*, 251–252. [CrossRef]
39. Bittker, S.S. Elevated Levels of 1,25-Dihydroxyvitamin D in Plasma as a Missing Risk Factor for Celiac Disease. *Clin. Exp. Gastroenterol.* **2020**, *13*, 1–15. [CrossRef]
40. Van Etten, E.; Mathieu, C. Immunoregulation by 1,25-Dihydroxyvitamin D3: Basic Concepts. *J. Steroid Biochem. Mol. Biol.* **2005**, *97*, 93–101. [CrossRef]
41. Xu, H.; Soruri, A.; Gieseler, R.K.H.; Peters, J.H. 1,25-Dihydroxyvitamin D3 Exerts Opposing Effects to IL-4 on MHC Class-II Antigen Expression, Accessory Activity, and Phagocytosis of Human Monocytes. *Scand. J. Immunol.* **1993**, *38*, 535–540. [CrossRef] [PubMed]
42. Penna, G.; Adorini, L. 1 Alpha,25-Dihydroxyvitamin D3 Inhibits Differentiation, Maturation, Activation, and Survival of Dendritic Cells Leading to Impaired Alloreactive T Cell Activation. *J. Immunol.* **2000**, *164*, 2405–2411. [CrossRef] [PubMed]
43. Pazár, B.; Ea, H.-K.; Narayan, S.; Kolly, L.; Bagnoud, N.; Chobaz, V.; Roger, T.; Lioté, F.; So, A.; Busso, N. Basic Calcium Phosphate Crystals Induce Monocyte/Macrophage IL-1β Secretion through the NLRP3 Inflammasome In Vitro. *J. Immunol.* **2011**, *186*, 2495–2502. [CrossRef] [PubMed]
44. Hakim, I.; Bar-Shavit, Z. Modulation of TNF-α Expression in Bone Marrow Macrophages: Involvement of Vitamin D Response Element. *J. Cell. Biochem.* **2003**, *88*, 986–998. [CrossRef]
45. Antal, A.S.; Dombrowski, Y.; Koglin, S.; Ruzicka, T.; Schauber, J. Impact of Vitamin D3 on Cutaneous Immunity and Antimicrobial Peptide Expression. *Dermato-Endocrinol.* **2011**, *3*, 18–22. [CrossRef]
46. Wang, T.-T.; Nestel, F.P.; Bourdeau, V.; Nagai, Y.; Wang, Q.; Liao, J.; Tavera-Mendoza, L.; Lin, R.; Hanrahan, J.W.; Mader, S.; et al. Cutting Edge: 1,25-Dihydroxyvitamin D3 Is a Direct Inducer of Antimicrobial Peptide Gene Expression. *J. Immunol.* **2004**, *173*, 2909–2912. [CrossRef]

47. Chernomordik, F.; Cercek, B.; Lio, W.M.; Mihailovic, P.M.; Yano, J.; Herscovici, R.; Zhao, X.; Zhou, J.; Chyu, K.-Y.; Shah, P.K.; et al. The Role of T Cells Reactive to the Cathelicidin Antimicrobial Peptide LL-37 in Acute Coronary Syndrome and Plaque Calcification. *Front. Immunol.* **2020**, *11*, 575577. [CrossRef]
48. Hruska, K.A.; Mathew, S.; Lund, R.; Qiu, P.; Pratt, R. Hyperphosphatemia of Chronic Kidney Disease. *Kidney Int.* **2008**, *74*, 148–157. [CrossRef]
49. Rapa, S.F.; Di Iorio, B.R.; Campiglia, P.; Heidland, A.; Marzocco, S. Inflammation and Oxidative Stress in Chronic Kidney Disease-Potential Therapeutic Role of Minerals, Vitamins and Plant-Derived Metabolites. *Int. J. Mol. Sci.* **2019**, *21*, 263. [CrossRef]
50. Chen, N.X.; O'Neill, K.; Akl, N.K.; Moe, S.M. Adipocyte Induced Arterial Calcification Is Prevented with Sodium Thiosulfate. *Biochem. Biophys. Res. Commun.* **2014**, *449*, 151–156. [CrossRef]
51. Adesso, S.; Popolo, A.; Bianco, G.; Sorrentino, R.; Pinto, A.; Autore, G.; Marzocco, S. The Uremic Toxin Indoxyl Sulphate Enhances Macrophage Response to LPS. *PLoS ONE* **2013**, *8*, e76778. [CrossRef] [PubMed]
52. Stockler-Pinto, M.B.; Saldanha, J.F.; Yi, D.; Mafra, D.; Fouque, D.; Soulage, C.O. The Uremic Toxin Indoxyl Sulfate Exacerbates Reactive Oxygen Species Production and Inflammation in 3T3-L1 Adipose Cells. *Free Radic. Res.* **2016**, *50*, 337–344. [CrossRef] [PubMed]
53. Jahnen-Dechent, W.; Heiss, A.; Schäfer, C.; Ketteler, M.; Towler, D.A. Fetuin-A Regulation of Calcified Matrix Metabolism. *Circ. Res.* **2011**, *108*, 1494–1509. [CrossRef]
54. Bjørklund, G.; Svanberg, E.; Dadar, M.; Card, D.J.; Chirumbolo, S.; Harrington, D.J.; Aaseth, J. The Role of Matrix Gla Protein (MGP) in Vascular Calcification. *Curr. Med. Chem.* **2020**, *27*, 1647–1660. [CrossRef] [PubMed]
55. Sowers, K.M.; Hayden, M.R. Calcific Uremic Arteriolopathy: Pathophysiology, Reactive Oxygen Species and Therapeutic Approaches. *Oxid. Med. Cell. Longev.* **2010**, *3*, 329291. [CrossRef]
56. Chung, M.P.; Richardson, C.; Kirakossian, D.; Orandi, A.B.; Saketkoo, L.A.; Rider, L.G.; Schiffenbauer, A.; von Mühlen, C.A.; Chung, L.; Assessment, I.M.; et al. Calcinosis Biomarkers in Adult and Juvenile Dermatomyositis. *Autoimmun. Rev.* **2020**, *19*, 102533. [CrossRef] [PubMed]
57. Balin, S.J.; Wetter, D.A.; Andersen, L.K.; Davis, M.D.P. Calcinosis Cutis Occurring in Association with Autoimmune Connective Tissue Disease: The Mayo Clinic Experience with 78 Patients, 1996–2009. *Arch. Dermatol.* **2012**, *148*, 455–462. [CrossRef]
58. Cruz-Domínguez, M.P.; García-Collinot, G.; Saavedra, M.A.; Medina, G.; Carranza-Muleiro, R.A.; Vera-Lastra, O.L.; Jara, L.J. Clinical, Biochemical, and Radiological Characterization of the Calcinosis in a Cohort of Mexican Patients with Systemic Sclerosis. *Clin. Rheumatol.* **2017**, *36*, 111–117. [CrossRef]
59. Morgan, N.D.; Shah, A.A.; Mayes, M.D.; Domsic, R.T.; Medsger, T.A., Jr.; Steen, V.D.; Varga, J.; Carns, M.; Ramos, P.S.; Silver, R.M.; et al. Clinical and Serological Features of Systemic Sclerosis in a Multicenter African American Cohort: Analysis of the Genome Research in African American Scleroderma Patients Clinical Database. *Medicine* **2017**, *96*, e8980. [CrossRef]
60. Baron, M.; Pope, J.; Robinson, D.; Jones, N.; Khalidi, N.; Docherty, P.; Kaminska, E.; Masetto, A.; Sutton, E.; Mathieu, J.-P.; et al. Calcinosis Is Associated with Digital Ischaemia in Systemic Sclerosis-a Longitudinal Study. *Rheumatology* **2016**, *55*, 2148–2155. [CrossRef]
61. Tolosa-Vilella, C.; Morera-Morales, M.L.; Simeón-Aznar, C.P.; Marí-Alfonso, B.; Colunga-Arguelles, D.; Callejas Rubio, J.L.; Rubio-Rivas, M.; Freire-Dapena, M.; Guillén-Del Castillo, A.; Iniesta-Arandia, N.; et al. Digital Ulcers and Cutaneous Subsets of Systemic Sclerosis: Clinical, Immunological, Nailfold Capillaroscopy, and Survival Differences in the Spanish RESCLE Registry. *Semin. Arthritis Rheum.* **2016**, *46*, 200–208. [CrossRef] [PubMed]
62. Gunawardena, H.; Betteridge, Z.E.; McHugh, N.J. Myositis-Specific Autoantibodies: Their Clinical and Pathogenic Significance in Disease Expression. *Rheumatology* **2009**, *48*, 607–612. [CrossRef] [PubMed]
63. Nombel, A.; Fabien, N.; Coutant, F. Dermatomyositis With Anti-MDA5 Antibodies: Bioclinical Features, Pathogenesis and Emerging Therapies. *Front. Immunol.* **2021**, *12*, 773352. [CrossRef] [PubMed]
64. Zahn, S.; Barchet, W.; Rehkämper, C.; Hornung, T.; Bieber, T.; Tüting, T.; Wenzel, J. Enhanced Skin Expression of Melanoma Differentiation-Associated Gene 5 (MDA5) in Dermatomyositis and Related Autoimmune Diseases. *J. Am. Acad. Dermatol.* **2011**, *64*, 988–989. [CrossRef] [PubMed]
65. Wenzel, J.; Tüting, T. An IFN-Associated Cytotoxic Cellular Immune Response against Viral, Self-, or Tumor Antigens Is a Common Pathogenetic Feature in "Interface Dermatitis". *J. Investig. Dermatol.* **2008**, *128*, 2392–2402. [CrossRef]
66. Valenzuela, A.; Chung, L.; Casciola-Rosen, L.; Fiorentino, D. Identification of Clinical Features and Autoantibodies Associated with Calcinosis in Dermatomyositis. *JAMA Dermatol.* **2014**, *150*, 724–729. [CrossRef]
67. Pisetsky, D.S. Immune Phenotypes in Individuals Positive for Antinuclear Antibodies: The Impact of Race and Ethnicity. *J. Allergy Clin. Immunol.* **2020**, *146*, 1346–1348. [CrossRef]
68. Duvvuri, B.; Pachman, L.; Moore, R.; Morgan, G.; Klein-Gitelman, M.; Curran, M.L.; Doty, S.; Lood, C. Mitochondrial Contribution to Juvenile Dermatomyositis Pathogenesis. In Proceedings of the Arthritis & Rheumatology, Hoboken, NJ, USA, 12 November 2019; Volume 71.
69. Glennon, N.B.; Jabado, O.; Lo, M.K.; Shaw, M.L. Transcriptome Profiling of the Virus-Induced Innate Immune Response in Pteropus Vampyrus and Its Attenuation by Nipah Virus Interferon Antagonist Functions. *J. Virol.* **2022**, *89*, 7550–7566. [CrossRef]
70. Paradowska-Gorycka, A. U1-RNP and Toll-like Receptors in the Pathogenesis of Mixed Connective Tissue DiseasePart II. Endosomal TLRs and Their Biological Significance in the Pathogenesis of Mixed Connective Tissue Disease. *Reumatologia* **2015**, *53*, 143–151. [CrossRef]

71. Gentili, M.; Lahaye, X.; Nadalin, F.; Nader, G.P.F.; Puig Lombardi, E.; Herve, S.; De Silva, N.S.; Rookhuizen, D.C.; Zueva, E.; Goudot, C.; et al. The N-Terminal Domain of CGAS Determines Preferential Association with Centromeric DNA and Innate Immune Activation in the Nucleus. *Cell Rep.* **2019**, *26*, 2377–2393.e13. [CrossRef]
72. Rialdi, A.; Campisi, L.; Zhao, N.; Lagda, A.C.; Pietzsch, C.; Ho, J.S.Y.; Martinez-Gil, L.; Fenouil, R.; Chen, X.; Edwards, M.; et al. Topoisomerase 1 Inhibition Suppresses Inflammatory Genes and Protects from Death by Inflammation. *Science* **2016**, *352*, aad7993. [CrossRef] [PubMed]
73. Pepin, G.; Nejad, C.; Ferrand, J.; Thomas, B.J.; Stunden, H.J.; Sanij, E.; Foo, C.-H.; Stewart, C.R.; Cain, J.E.; Bardin, P.G.; et al. Topoisomerase 1 Inhibition Promotes Cyclic GMP-AMP Synthase-Dependent Antiviral Responses. *mBio* **2017**, *8*, e01611-17. [CrossRef] [PubMed]
74. Gunawardena, H.; Wedderburn, L.R.; North, J.; Betteridge, Z.; Dunphy, J.; Chinoy, H.; Davidson, J.E.; Cooper, R.G.; McHugh, N.J.; Juvenile Dermatomyositis Research Group UK. Clinical Associations of Autoantibodies to a P155/140 KDa Doublet Protein in Juvenile Dermatomyositis. *Rheumatology* **2008**, *47*, 324–328. [CrossRef] [PubMed]
75. Geoffroy, M.-C.; Chelbi-Alix, M.K. Role of Promyelocytic Leukemia Protein in Host Antiviral Defense. *J. Interf. Cytokine Res. Off. J. Int. Soc. Interf. Cytokine Res.* **2011**, *31*, 145–158. [CrossRef] [PubMed]
76. Gaur, P.S.; Naveen, R.; Anuja, A.K.; Singh, M.K.; Rai, M.K.; Muhammed, R.; Sahu, A.K.; Agarwal, V.; Gupta, L. Anti-Mitochondrial Antibodies in Indian Patients with Idiopathic Inflammatory Myopathies. *Int. J. Rheum. Dis.* **2022**, *25*, 659–668. [CrossRef] [PubMed]
77. Björn, I.; Daniel, S.; Tobias, S.; Anita, S.; Ann-Charlotte, B.; Vesa, L.; Kourosh, L.; Mårten, S.; Giannis, S.; Anders, R. Lymphocytes Eject Interferogenic Mitochondrial DNA Webs in Response to CpG and Non-CpG Oligodeoxynucleotides of Class C. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, E478–E487. [CrossRef]
78. Pustylnikov, S.; Costabile, F.; Beghi, S.; Facciabene, A. Targeting Mitochondria in Cancer: Current Concepts and Immunotherapy Approaches. *Transl. Res.* **2018**, *202*, 35–51. [CrossRef]
79. Fritzler, M.J.; Kinsella, T.D. The CREST Syndrome: A Distinct Serologic Entity with Anticentromere Antibodies. *Am. J. Med.* **1980**, *69*, 520–526. [CrossRef]
80. Kajio, N.; Takeshita, M.; Suzuki, K.; Kaneda, Y.; Yamane, H.; Ikeura, K.; Sato, H.; Kato, S.; Shimizu, H.; Tsunoda, K.; et al. Anti-Centromere Antibodies Target Centromere–Kinetochore Macrocomplex: A Comprehensive Autoantigen Profiling. *Ann. Rheum. Dis.* **2021**, *80*, 651–659. [CrossRef]
81. Saardi, K.M.; Rosenstein, R.K.; Anadkat, M.J.; Micheletti, R.G.; Schiffenbauer, A.I.; Pavletic, S.Z.; Cowen, E.W. Calcinosis Cutis in the Setting of Chronic Skin Graft-Versus-Host Disease. *JAMA Dermatol.* **2020**, *156*, 814–817. [CrossRef]
82. Tsuchida, Y.; Sumitomo, S.; Fujio, K.; Yamamoto, K. Massive Calcinosis Cutis Associated with Primary Sjögren's Syndrome. *BMJ Case Rep.* **2016**, *2016*, bcr2015214006. [CrossRef] [PubMed]
83. Eastham, A.B.; Velez, N.F.; Chesebro, A.L.; Townsend, H.B.; Vleugels, R.A. Diffuse Dystrophic Calcinosis Cutis of the Scalp in a Patient with Scalp Discoid Lupus Erythematosus and Systemic Lupus Erythematosus. *JAMA Dermatol.* **2013**, *149*, 246–248. [CrossRef] [PubMed]
84. Charles, N.; Hardwick, D.; Daugas, E.; Illei, G.G.; Rivera, J. Basophils and the T Helper 2 Environment Can Promote the Development of Lupus Nephritis. *Nat. Med.* **2010**, *16*, 701–707. [CrossRef] [PubMed]
85. Itoh, O.; Nishimaki, T.; Itoh, M.; Ohira, H.; Irisawa, A.; Kaise, S.; Kasukawa, R. Mixed Connective Tissue Disease with Severe Pulmonary Hypertension and Extensive Subcutaneous Calcification. *Intern. Med.* **1998**, *37*, 421–425. [CrossRef]
86. Goolamali, S.I.; Gordon, P.; Salisbury, J.; Creamer, D. Subcutaneous Calcification Presenting in a Patient with Mixed Connective Tissue Disease and Cutaneous Polyarteritis Nodosa. *Clin. Exp. Dermatol.* **2009**, *34*, e141–e144. [CrossRef]
87. Ashraf, M.; Gopikrishnan, K.; Umamahesvaran, B.; Sambandam, S.N. Presentation of Calcinosis Cutis Universalis in Mixed Connective Tissue Disorder: An Encounter during Hip Arthroplasty. *BMJ Case Rep.* **2017**, *2017*, bcr2017219278. [CrossRef]
88. Greidinger, E.L.; Zang, Y.; Jaimes, K.; Hogenmiller, S.; Nassiri, M.; Bejarano, P.; Barber, G.N.; Hoffman, R.W. A Murine Model of Mixed Connective Tissue Disease Induced with U1 Small Nuclear RNP Autoantigen. *Arthritis Rheum.* **2006**, *54*, 661–669. [CrossRef]
89. Drake, M.T.; Clarke, B.L.; Khosla, S. Bisphosphonates: Mechanism of Action and Role in Clinical Practice. *Mayo Clin. Proc.* **2008**, *83*, 1032–1045. [CrossRef]
90. Tayfur, A.C.; Topaloglu, R.; Gulhan, B.; Bilginer, Y. Bisphosphonates in Juvenile Dermatomyositis with Dystrophic Calcinosis. *Mod. Rheumatol.* **2015**, *25*, 615–620. [CrossRef]
91. Saini, I.; Kalaivani, M.; Kabra, S.K. Calcinosis in Juvenile Dermatomyositis: Frequency, Risk Factors and Outcome. *Rheumatol. Int.* **2016**, *36*, 961–965. [CrossRef]
92. Metzger, A.L.; Singer, F.R.; Bluestone, R.; Pearson, C.M. Failure of Disodium Etidronate in Calcinosis Due to Dermatomyositis and Scleroderma. *N. Engl. J. Med.* **1974**, *291*, 1294–1296. [CrossRef] [PubMed]
93. Patntirapong, S.; Phupunporn, P.; Vanichtantiphong, D.; Thanetchaloempong, W. Inhibition of Macrophage Viability by Bound and Free Bisphosphonates. *Acta Histochem.* **2019**, *121*, 400–406. [CrossRef] [PubMed]
94. Thompson, K.; Rogers, M.J.; Coxon, F.P.; Crockett, J.C. Cytosolic Entry of Bisphosphonate Drugs Requires Acidification of Vesicles after Fluid-Phase Endocytosis. *Mol. Pharmacol.* **2006**, *69*, 1624–1632. [CrossRef] [PubMed]
95. Rogers, T.L.; Holen, I. Tumour Macrophages as Potential Targets of Bisphosphonates. *J. Transl. Med.* **2011**, *9*, 177. [CrossRef] [PubMed]

96. Schmidt, K.E.; Kuepper, J.M.; Schumak, B.; Alferink, J.; Hofmann, A.; Howland, S.W.; Renia, L.; Limmer, A.; Specht, S.; Hoerauf, A. Doxycycline Inhibits Experimental Cerebral Malaria by Reducing Inflammatory Immune Reactions and Tissue-Degrading Mediators. *PLoS ONE* **2018**, *13*, e0192717. [CrossRef] [PubMed]
97. Sun, J.; Shigemi, H.; Tanaka, Y.; Yamauchi, T.; Ueda, T.; Iwasaki, H. Tetracyclines Downregulate the Production of LPS-Induced Cytokines and Chemokines in THP-1 Cells via ERK, P38, and Nuclear Factor-KB Signaling Pathways. *Biochem. Biophys. Rep.* **2015**, *4*, 397–404. [CrossRef]
98. Reiter, N.; El-Shabrawi, L.; Leinweber, B.; Berghold, A.; Aberer, E. Calcinosis Cutis: Part II. Treatment Options. *J. Am. Acad. Dermatol.* **2011**, *65*, 15–22. [CrossRef]
99. Leung, Y.Y.; Yao Hui, L.L.; Kraus, V.B. Colchicine—Update on Mechanisms of Action and Therapeutic Uses. *Semin. Arthritis Rheum.* **2015**, *45*, 341–350. [CrossRef]
100. Fuchs, D.; Fruchter, L.; Fishel, B.; Holtzman, M.; Yaron, M. Colchicine Suppression of Local Inflammation Due to Calcinosis in Dermatomyositis and Progressive Systemic Sclerosis. *Clin. Rheumatol.* **1986**, *5*, 527–530.
101. Stahn, C.; Löwenberg, M.; Hommes, D.W.; Buttgereit, F. Molecular Mechanisms of Glucocorticoid Action and Selective Glucocorticoid Receptor Agonists. *Mol. Cell. Endocrinol.* **2007**, *275*, 71–78. [CrossRef]
102. Ledon, J.A.; Savas, J.; Franca, K.; Chacon, A.; Nouri, K. Intralesional Treatment for Keloids and Hypertrophic Scars: A Review. *Dermatol. Surg.* **2013**, *39*, 1745–1757. [CrossRef] [PubMed]
103. Quinti, I.; Mitrevski, M. Modulatory Effects of Antibody Replacement Therapy to Innate and Adaptive Immune Cells. *Front. Immunol.* **2017**, *8*, 697. [CrossRef] [PubMed]
104. Bayry, J.; Lacroix-Desmazes, S.; Carbonneil, C.; Misra, N.; Donkova, V.; Pashov, A.; Chevailler, A.; Mouthon, L.; Weill, B.; Bruneval, P.; et al. Inhibition of Maturation and Function of Dendritic Cells by Intravenous Immunoglobulin. *Blood* **2003**, *101*, 758–765. [CrossRef] [PubMed]
105. Xie, F.; Williams, P.; Batchelor, R.; Downs, A.; Haigh, R. Successful Treatment of Dermatomyositis and Associated Calcinosis with Adalimumab. *Clin. Exp. Dermatol.* **2020**, *45*, 945–949. [CrossRef] [PubMed]
106. Tosounidou, S.; MacDonald, H.; Situnayake, D. Successful Treatment of Calcinosis with Infliximab in a Patient with Systemic Sclerosis/Myositis Overlap Syndrome. *Rheumatology* **2014**, *53*, 960–961. [CrossRef]
107. Robertson, L.; Marshall, R.; Hickling, P. Treatment Cutaneous Calcinosis in Limited Systemic Sclerosis with Minocyclin. *Ann. Rheum. Dis.* **2003**, *62*, 267–269. [CrossRef]
108. Cho, D.-H.; Lee, W.-H.; Park, S.-J. Treatment of Calcinosis Cutis with Minocycline in Five Dogs. *J. Vet. Clin.* **2017**, *34*, 119–122. [CrossRef]
109. Reiter, N.; El-Shabrawi, L.; Leinweber, B.; Aberer, E. Subcutaneous Morphea with Dystrophic Calcification with Response to Ceftriaxone Treatment. *J. Am. Acad. Dermatol.* **2010**, *63*, e53–e55. [CrossRef]
110. Ochoa-Aguilar, A.; Ventura-Martinez, R.; Sotomayor-Sobrino, M.A.; Jaimez, R.; Coffeen, U.; Jiménez-González, A.; Balcázar-Ochoa, L.G.; Pérez-Medina-Carballo, R.; Rodriguez, R.; Plancarte-Sánchez, R. Ceftriaxone and Clavulanic Acid Induce Antiallodynia and Anti-Inflammatory Effects in Rats Using the Carrageenan Model. *J. Pain Res.* **2018**, *11*, 977–985. [CrossRef]
111. Sanchez, J.; Le Jan, S.; Muller, C.; François, C.; Renard, Y.; Durlach, A.; Bernard, P.; Reguiai, Z.; Antonicelli, F. Matrix Remodelling and MMP Expression/Activation Are Associated with Hidradenitis Suppurativa Skin Inflammation. *Exp. Dermatol.* **2019**, *28*, 593–600. [CrossRef]
112. Parks, W.C.; Wilson, C.L.; López-Boado, Y.S. Matrix Metalloproteinases as Modulators of Inflammation and Innate Immunity. *Nat. Rev. Immunol.* **2004**, *4*, 617–629. [CrossRef] [PubMed]
113. Chen, N.X.; O'Neill, K.D.; Chen, X.; Kiattisunthorn, K.; Gattone, V.H.; Moe, S.M. Activation of Arterial Matrix Metalloproteinases Leads to Vascular Calcification in Chronic Kidney Disease. *Am. J. Nephrol.* **2011**, *34*, 211–219. [CrossRef]
114. Fredi, M.; Bartoli, F.; Cavazzana, I.; Ceribelli, A.; Carabellese, N.; Tincani, A.; Satoh, M.; Franceschini, F. Calcinosis in Poly-Dermatomyositis: Clinical and Laboratory Predictors and Treatment Options. *Clin. Exp. Rheumatol.* **2017**, *35*, 303–308. [PubMed]
115. Dupuis, J.; Sirois, M.G.; Rhéaume, E.; Nguyen, Q.T.; Clavet-Lanthier, M.-É.; Brand, G.; Mihalache-Avram, T.; Théberge-Julien, G.; Charpentier, D.; Rhainds, D.; et al. Colchicine Reduces Lung Injury in Experimental Acute Respiratory Distress Syndrome. *PLoS ONE* **2020**, *15*, e0242318. [CrossRef] [PubMed]
116. Vaidya, K.; Tucker, B.; Kurup, R.; Khandkar, C.; Pandzic, E.; Barraclough, J.; Machet, J.; Misra, A.; Kavurma, M.; Martinez, G.; et al. Colchicine Inhibits Neutrophil Extracellular Trap Formation in Patients with Acute Coronary Syndrome after Percutaneous Coronary Intervention. *J. Am. Heart Assoc.* **2021**, *10*, e018993. [CrossRef]
117. Lee, S.S.; Felsenstein, J.; Tanzer, F.R. Calcinosis Cutis Circumscripta: Treatment with an Intralesional Corticosteroid. *Arch. Dermatol.* **1978**, *114*, 1080–1081. [CrossRef]
118. Hazen, P.G.; Walker, A.E.; Carney, J.F.; Stewart, J.J. Cutaneous Calcinosis of Scleroderma: Successful Treatment with Intralesional Adrenal Steroids. *Arch. Dermatol.* **1982**, *118*, 366–367. [CrossRef]
119. Al-Mayouf, S.M.; Alsonbul, A.; Alismail, K. Localized Calcinosis in Juvenile Dermatomyositis: Successful Treatment with Intralesional Corticosteroids Injection. *Int. J. Rheum. Dis.* **2010**, *13*, e26–e28. [CrossRef]
120. Schanz, S.; Ulmer, A.; Fierlbeck, G. Response of Dystrophic Calcification to Intravenous Immunoglobulin. *Arch. Dermatol.* **2008**, *144*, 585–587. [CrossRef]
121. Peñate, Y.; Guillermo, N.; Melwani, P.; Martel, R.; Hernández-Machín, B.; Borrego, L. Calcinosis Cutis Associated with Amyopathic Dermatomyositis: Response to Intravenous Immunoglobulin. *J. Am. Acad. Dermatol.* **2009**, *60*, 1076–1077. [CrossRef]

122. Kalajian, A.H.; Perryman, J.H.; Callen, J.P. Intravenous Immunoglobulin Therapy for Dystrophic Calcinosis Cutis: Unreliable in Our Hands. *Arch. Dermatol.* **2009**, *145*, 334. [CrossRef] [PubMed]
123. Galimberti, F.; Li, Y.; Fernandez, A.P. Intravenous Immunoglobulin for Treatment of Dermatomyositis-Associated Dystrophic Calcinosis. *J. Am. Acad. Dermatol.* **2015**, *73*, 174–176. [CrossRef] [PubMed]
124. Von Gunten, S.; Simon, H.-U. Cell Death Modulation by Intravenous Immunoglobulin. *J. Clin. Immunol.* **2010**, *30* (Suppl. 1), S24–S30. [CrossRef] [PubMed]
125. Giuggioli, D.; Lumetti, F.; Colaci, M.; Fallahi, P.; Antonelli, A.; Ferri, C. Rituximab in the Treatment of Patients with Systemic Sclerosis. Our Experience and Review of the Literature. *Autoimmun. Rev.* **2015**, *14*, 1072–1078. [CrossRef] [PubMed]
126. Moazedi-Fuerst, F.C.; Kielhauser, S.M.; Bodo, K.; Graninger, W.B. Dosage of Rituximab in Systemic Sclerosis: 2-Year Results of Five Cases. *Clin. Exp. Dermatol.* **2015**, *40*, 211–212. [CrossRef] [PubMed]
127. Bader-Meunier, B.; Decaluwe, H.; Barnerias, C.; Gherardi, R.; Quartier, P.; Faye, A.; Guigonis, V.; Pagnier, A.; Brochard, K.; Sibilia, J.; et al. Safety and Efficacy of Rituximab in Severe Juvenile Dermatomyositis: Results from 9 Patients from the French Autoimmunity and Rituximab Registry. *J. Rheumatol.* **2011**, *38*, 1436–1440. [CrossRef] [PubMed]
128. Aggarwal, R.; Loganathan, P.; Koontz, D.; Qi, Z.; Reed, A.M.; Oddis, C. V Cutaneous Improvement in Refractory Adult and Juvenile Dermatomyositis after Treatment with Rituximab. *Rheumatology* **2017**, *56*, 247–254. [CrossRef]
129. Miyagawa, I.; Nakayamada, S.; Nakano, K.; Kubo, S.; Iwata, S.; Miyazaki, Y.; Yoshikawa, M.; Yoshinari, H.; Tanaka, Y. Precision Medicine Using Different Biological DMARDs Based on Characteristic Phenotypes of Peripheral T Helper Cells in Psoriatic Arthritis. *Rheumatology* **2019**, *58*, 336–344. [CrossRef]
130. Throm, A.A.; Alinger, J.B.; Pingel, J.T.; Daugherty, A.L.; Pachman, L.M.; French, A.R. Dysregulated NK Cell PLCγ2 Signaling and Activity in Juvenile Dermatomyositis. *JCI Insight* **2018**, *3*, e123236. [CrossRef]