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CME REVIEW

Copa Syndrome: a Novel Autosomal Dominant Immune Dysregulatory Disease

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Abstract Inherently defective immunity typically results in either ineffective host defense, immune regulation, or both. As a category of primary immunodeficiency diseases, those that impair immune regulation can lead to autoimmunity and/or autoinflammation. In this review we focus on one of the most recently discovered primary immunodeficiencies that leads to immune dysregulation: "Copa syndrome". Copa syndrome is named for the gene mutated in the disease, which encodes the alpha subunit of the coatomer complex-I that, in aggregate, is devoted to transiting molecular cargo from the Golgi complex to the endoplasmic reticulum (ER). Copa syndrome is autosomal dominant with variable expressivity and results from mutations affecting a narrow amino acid stretch in the *COPA* gene-encoding COPα protein. Patients with these mutations typically develop arthritis and interstitial lung disease with pulmonary hemorrhage representing a striking feature. Immunologically Copa syndrome is associated with autoantibody development, increased Th17 cells and pro-inflammatory cytokine expression including IL-1β and IL-6. Insights have also been gained into the underlying mechanism of Copa syndrome, which include excessive ER stress owing to the impaired return of proteins from the Golgi, and presumably resulting aberrant cellular autophagy. As such it represents a novel cellular disorder of intracellular trafficking associated with a specific clinical presentation and phenotype.

Keywords Autoimmunity · interstitial lung disease · arthritis · autosomal dominant · primary immunodeficiency

Introduction

Genetic immune dysregulatory syndromes are classified by the International Union of Immunological Societies (IUIS) as primary immunodeficiency diseases [1]. In recent decades, improved understanding of these inherited pediatric immune dysregulatory syndromes have led to important clinical and biological discoveries. The 1982 description of what is now known as immune dysregulation, polyendocrinopathy, and enteropathy, X-linked (IPEX), allowed for the identification of causative FOXP3 mutations and deep insights into the role of human regulatory T cells in the suppression of autoimmunity. Also one of the most well described autoimmune/immune dysregulatory genetic disorders, Autoimmune Lymphoproliferative Syndrome (ALPS), led to the understanding of Fas/FasL regulated mechanisms of apoptosis. Similarly, the recognition of the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) syndrome led to the discovery of the autoimmune regulator (AIRE) gene and its essential function in thymic self-antigen presentation. Additional single gene, heritable causes of immune perturbation and deficiency of immune regulatory or control mechanisms continue to accumulate [2]. While rare, these genetic diseases have provided extraordinary perspective into basic immunological mechanisms. Ideally these, in turn give rise to more specific therapies for these

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patients and to tangential benefit for other patients affected by disorders related to these immunological mechanisms.

Typically, pediatric autoimmune diseases affect non-immune tissues, such as joints, skin and kidneys as a feature of misdirected immunity. Other common autoimmune targets in children include endocrine organs (e.g., type 1 diabetes) and hematologic cells themselves (e.g., immune-mediated cytopenias). However, pulmonary disease represents a relatively rare manifestation of immune dysregulation and autoimmunity in children and is seen in only a small number of phenotypic and genotypic conditions. The existence of immune-mediated pulmonary hemorrhage in children therefore points toward the presence of an underlying novel immune dysregulatory syndrome. When present in families it raises the possibility of, and opportunity for, a novel etiologic genetic discovery.

When severe, pulmonary autoimmunity can cause pulmonary hemorrhage. Examples of established causes of autoimmune pulmonary hemorrhage include granulomatosis with polyangiitis and microscopic polyangiitis, which are collectively referred to as the ANCA-associated vasculitidies. In these diseases, autoantibodies directed against neutrophil granule components myeloperoxidase and proteinase-3 cause inflammation and destruction of pulmonary capillaries and small vessels, leading to pulmonary hemorrhage [3]. Children with ANCA-associated vasculitis often present with fever, malaise, and renal disease in addition to pulmonary hemorrhage [4–6]. Systemic lupus erythematosis (SLE) has also been recognized as a cause of immune mediated pulmonary hemorrhage in children and has been associated with a variety of immunological aberrations and associations [7]. SLE, along with other immune dysregulatory diseases such as juvenile dermatomyosits and scleroderma, can lead to other forms of pulmonary disease, most notably interstitial lung diseases including non-specific interstitial pneumonia (NSIP) [8–10]. Nonetheless, although some families demonstrate increased risk for ANCA-associated vasculitidies and SLE, no known singular genetic cause for even a subset of these conditions has been demonstrated.

Recent advances have begun to provide both insights and mechanistic clues. The 2014 description of gain of function mutations in transmembrane protein 173 (TMEM173), also known as stimulator of interferon genes (STING) represent a first major advance [11, 12]. Mutations in the TMEM173 gene result in STING-associated vasculopathy with onset in infancy (SAVI). The presenting finding in these patients is typically cutaneous with later onset pulmonary disease, primarily interstitial lung disease. Mechanistically they have impaired intracellular DNA sensing leading to substantively increased production of and inflammation resulting from type-I interferon. While complex and mechanistically informative, SAVI does have many characteristics of an early onset lupus-like syndrome but does not typically result in pulmonary hemorrhage as a presenting feature.

We have recently identified a novel autosomal dominant syndrome consisting of autoimmune lung, joint, and kidney disease in pediatric patients that is caused by mutations in the coatomer associated protein subunit alpha (COPα) gene (*COPA*; http://www.omim.org/entry/601924) [13, 14]. This "Copa syndrome" is caused by immune dysregulation, resulting in diffuse alveolar hemorrhage or interstitial lung disease, arthritis, and renal disease. COPα is part of the coatomer protein complex I (COPI) and helps to mediate retrograde movement of vesicles from the Golgi to endoplasmic reticulum (ER). These cellular processes were not previously recognized as causes of immune-mediated disease. Thus, Copa syndrome promises to yield important new insights concerning the role of intracellular trafficking in immune regulation and further mechanistic study will hopefully give rise to new therapeutic strategies to combat immune dysregulation. Interestingly COPA is expressed in all cell types while the clinical presentations are limited to pulmonary, joint, and renal tissues. Presumably it may be that these tissues are either more sensitive to the mutation and its impact on intracellular traffic, or they are more sensitive to the resulting pro-inflammatory environment which these mutations induce. In this summary, we review the clinical features of Copa syndrome and propose potential molecular mechanisms for the role of *COPA* mutations in the generation of immune dysregulation.

Clinical Phenotype

Copa syndrome is inherited in an autosomal dominant pattern with variable penetrance. Clinical features of the disease are shown in Table 1 and are compared to other major known immune-mediated pulmonary hemorrhage and related syndromes. Patients with Copa syndrome develop pulmonary symptoms (most frequently pulmonary hemorrhage), arthritis, and renal disease. Most present early in life with 76 % exhibiting signs and symptoms of disease under 5 years of age. The most common presenting symptoms include cough and tachypnea with some patients requiring supplemental oxygen at a young age. A female predominance for penetrance appears to exist, but a larger cohort of patients is needed to confirm this observation. The following sections delineate what is known about the clinical aspects of Copa syndrome based upon the first five families reported in the initial report of this disease and in light of other as of yet unreported patients presently under investigation. Given that Copa syndrome is autosomal dominant and that a relatively large number of patients were identified within a relatively short period of time, we would predict that it is not extremely rare. The diversity of this phenotype represents a rapidly evolving story.

Table 1 Clinical Features of COPA Syndrome and Other Immune-mediated Pulmonary Hemorrhage Related Syndromes

	Associated Gene	Inheritance pattern	Pulmonary Hemorrhage	Renal Disease	Arthritis	GGO on chest CT	Cysts on chest CT	Other ILD	Skin Disease
Copa Syndrome	*COPA*	AD	+++	++++	+++	+++	+++	++	+
ANCA-associated vasculitis	NA	NA	++++	+++	+	++++	−	−	+
SLE	NA	NA	+	++++	++++	+++	−	++++	+++
SAVI-syndrome	*TMEM173*	AD	−	+	−	+++	−	+++	++++

Copa cotamer associated protein a, *ANCA* anti-neutrophil cytoplasmic antibody, *SLE* systemic lupus erythematosis, *SAVI* Stimulator of interferon genes–associated vasculopathy with onset in infancy, *AD* autosomal dominant, *NA* not applicable, *ILD* interstitial lung disease, *GGO* ground glass opacities

Immune Dysregulation

Copa syndrome is marked by immune dysregulation, which can present in various forms. Approximately 80 % of patients develop a positive ANA titer. Homogenous, speckled, and diffuse patterns of ANA staining have all been reported (with titers measured as high as 1:1,280). Other autoantibodies that may be present include both cytoplasmic anti-neutrophil cytoplasmic antibiody (cANCA) and perinuclear anti neutrophil cytoplasmic antibody (pANCA), anti-myeloperoxidase antibodies, anti-proteinase 3 antibodies, and rheumatoid factor antibodies. The presence and titers of antibodies vary with time and disease activity. However, no single autoantibody has yet emerged as a marker of disease severity, disease activity or disease progression. The full spectrum of autoantibodies in Copa syndrome is presently unknown, but is being pursued through multiple antigen screening approaches. Patients also often have elevated levels of serum markers of inflammation, including C-reactive protein and erythrocyte sedimentation rate.

In Copa syndrome, changes in T cell populations are observed that would be expected to promote immune dysregulation and autoimmunity. Patients typically have normal numbers and percentages of lymphocytes and lymphocyte subsets along with unremarkable immunoglobulin levels and intact production of specific antibodies. However, $CD4^+$ T cells show significant skewing toward T_H17 phenotype, which have been implicated in autoimmunity. Flow cytometry analyses reveal a significant increase in T_H17 cells with a comparable reduction in T_H1 cells in patients compared to healthy controls. Copa syndrome patients are also known to have increased expression (at the mRNA level) of T_H17 cell stimulating cytokines IL-1β, IL-6, and IL-23 (which represents at least a partial overlap with SAVI). Intriguingly, they demonstrate increased numbers of these potentially T_H17-skewed $CD4^+$ T cells within affected tissues thus likely implicating a role for these cells in disease pathogenesis. It is both curious and compelling that despite the skewing towards T_H17, Copa syndrome patients identified to date do not demonstrate psoriasis or inflammatory bowel disease both of which are manifestations of T_H17-associated disease. This speaks to the complexity of the IL-23/IL-17 axis and suggests that Copa syndrome presents an opportunity to refine our understanding of the human organ-specific roles of T_H17 cells and their regulation.

Based on the clinical phenotype a reasonable question is whether Copa syndrome is autoimmune or autoinflammatory. The most reasonable answer given the presently available data is both. As discussed below there are defective cellular mechanisms in Copa syndrome that can promote autoinflammation and inappropriate development of autoreactivity. While further experimental work will delineate which immune mechanism is predominant, it is likely that clinical approaches to patients are to be best served by maintaining focus upon both. It is also important to note that *COPA* is expressed both within and outside of the immune system and that the clinical phenotype is most likely a feature of aberrant COPI functions in both immunologic and somatic cells.

Pulmonary Disease

Pulmonary disease is universally present in known patients affected by Copa syndrome. Patients develop progressive lung disease with worsening pulmonary function. Pulmonary function testing demonstrates restriction with a symmetrically low forced vital capacity and forced expiratory volume in the first second. Plethysmography reveals a low total lung capacity, and when tested, diffusion capacity of carbon monoxide is low. The two most common manifestations of pulmonary disease that produce these changes in lung function include immune-mediated diffuse alveolar hemorrhage and interstitial lung disease.

Pulmonary hemorrhage generally occurs early in life with most patients identified thus far presenting with at least some findings before age 5, and can be identified in many patients through direct testing. Hemorrhage may be insidious, and not all patients report hemoptysis. However, some patients can develop life threatening pulmonary hemorrhage that requires endotracheal intubation, assisted ventilation and aggressive therapy with immunosuppression. When performed, bronchoscopy typically demonstrates a substantive increase in hemosiderin laden macrophages, consistent with diffuse alveolar hemorrhage. Chest x-rays are frequently notable for the presence of

diffuse alveolar opacities. CT scans of the chest exhibit a unique pattern marked by diffuse ground glass opacities with septal thickening and cyst formation (Fig. 1). Furthermore, the chest CT scan pattern changes with time in terms of increased cyst formation and decreased appearance of ground glass opacities. This pattern may resemble other causes of alveolar hemorrhage, but important distinctions exist. First, the ground glass opacities can be minimal and patchy. Second, cyst formation is not typically seen in alveolar hemorrhage and may distinguish Copa syndrome patients from others with similar diseases, including SAVI syndrome. The CT pattern is more consistent with non-specific interstitial pneumonia or lymphocytic interstitial pneumonia, patterns seen in immune dysregulatory syndromes, such as juvenile dermatomyositis and systemic sclerosis. The other immune dysregulatory syndromes do not have alveolar hemorrhage as a consistent feature, also distinguishing Copa syndrome from other known diseases. Histopathologically, increased red blood cells and hemosiderin laden macrophages are present in the alveolar spaces. Signs of pulmonary capillaritis are evident in most subjects with necrosis of the capillary walls. Neutrophils are often identified along the capillaries, consistent with immune-mediated hemorrhage (Fig. 2). A second, less common hispathologic pattern is seen with lymphoid aggregates around airways and features of pulmonary hemorrhage without capillaritis (Fig. 2).

Patients with Copa syndrome also develop interstitial lung disease. Interstitial lung disease has a later age of onset with most children presenting in their second decade of life. Lung biopsies of pediatric patients show both non-specific lymphocytic interstitial pneumonia and follicular bronchiolitis. Immunohistochemistry staining for B and T cells demonstrates the presence of $CD20^+$ cells, some $CD8^+$ cells, and notable numbers of $CD4^+$ T cells. Lymphocytic interstitial pneumonia is a known complication of common variable immunodeficiency disease, STAT3 gain of function mutations, CTLA4 mutations [15, 16] and *LRBA* deficiency [17, 18]. Interestingly, similar to Copa syndrome, *LRBA* deficiency causes abnormal cellular trafficking, with *LRBA* deficiency leading to increased lysosomal destruction of CTLA-4, immune dysregulation, and interstitial lung disease. Importantly, *LRBA* deficiency does not seem to cause pulmonary hemorrhage, thus distinguishing it to some degree from Copa syndrome. While both disorders have clear impacts on intracellular trafficking the nuances of the specific trafficking abnormalities clearly equate to major mechanistic and phenotypic differences. Follicular bronchiolitis has also been associated with immune dysregulation [19]. At least one patient with lymphocytic interstitial pneumonia has developed pulmonary hemorrhage. Diffuse interstitial pulmonary neuroendocrine cell hyperplasia (DIPNECH), a recently described, rare interstitial lung disease in adults [20] was seen in two related adult patients with Copa syndrome. Both patients have severe lung disease, and one has required lung transplantation. It remains unclear whether additional Copa syndrome patients will develop DIPNECH over time. It is also unclear as to what unifying immunological or lung parenchymal mechanisms equate to this collection of pulmonary findings. Additional exploration of these conditions from this vantage will likely provide insight. Finally, it is unclear as to what pulmonary components of Copa syndrome are a feature of inherent lung parenchymal cell abnormalities vs immunologic contributions. As there are ER stress abnormalities demonstrated in pulmonary

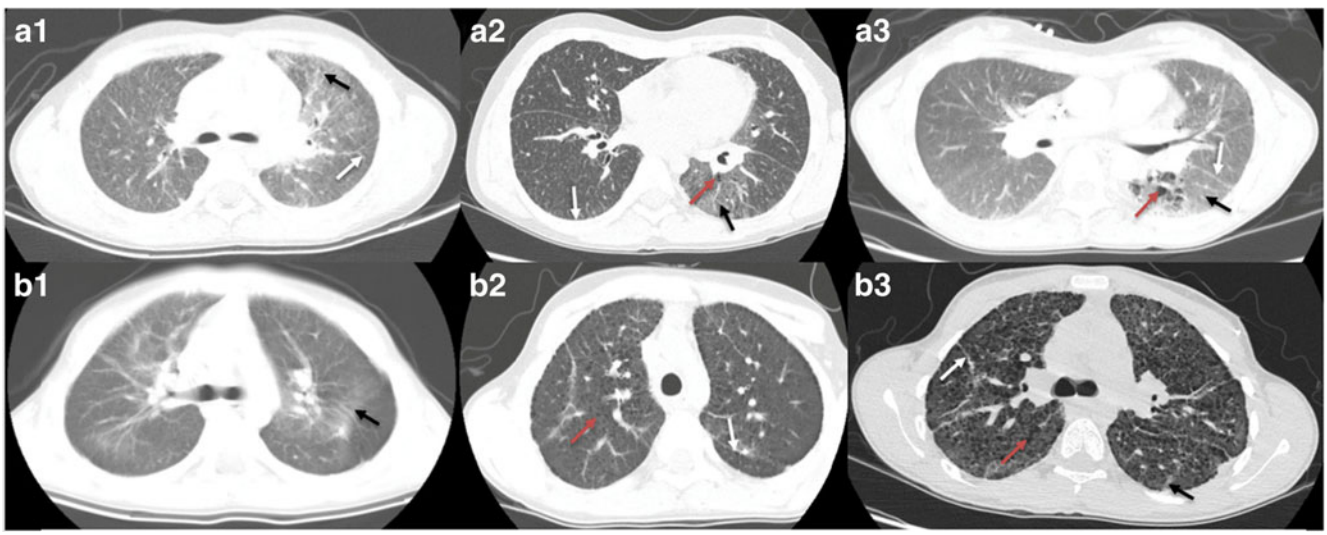

Fig. 1 Lung imaging findings over time in Copa patients. Serial chest CT images from two patients (A and B) with Copa syndrome. Images initially show ground glass opacities (*black arrows*) and septal thickening (*white arrows*) with progression to cysts (*red arrows*). Figure A1 was at 5 years of age, A2 at 11, and A3 at 14. Figure B1 was at 5 years of age, B2 at 12 years of age, and B3 at 17

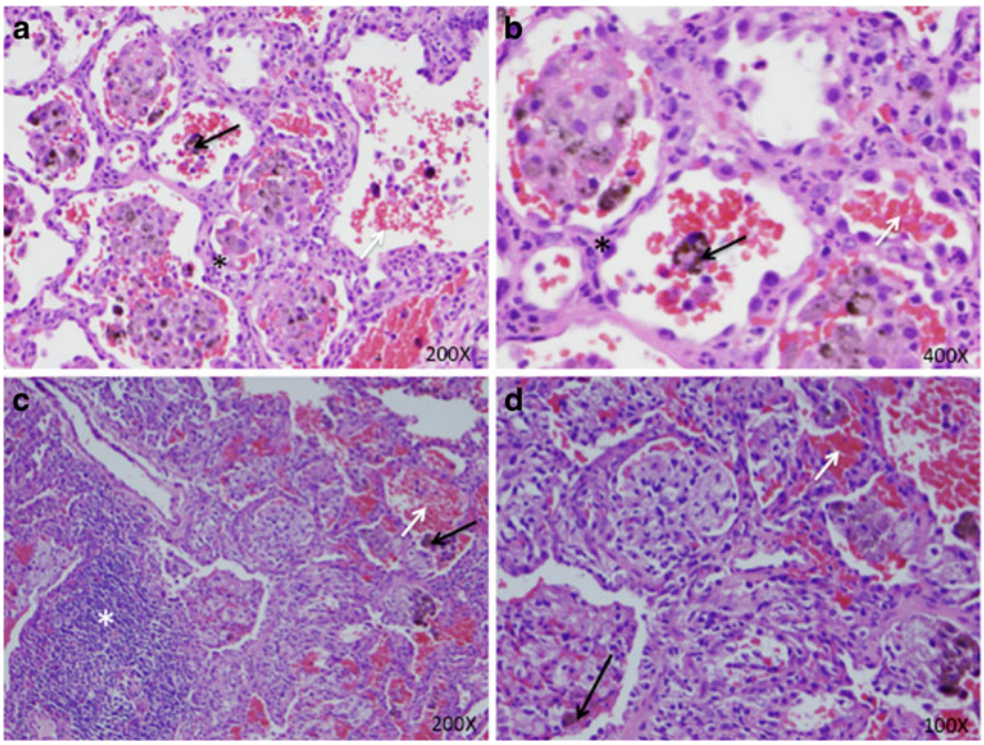

Fig. 2 Lung histopathology from Copa syndrome patients. Figures **a** and **b** show acute hemorrhage (*white arrows*) with evidence of chronic hemorrhage with hemosiderin laden macrophages (*black arrows*). There is also extensive capillaritis noted with neutrophils in the alveolar septa along the pulmonary capillaries (black asterisk). Figures **c** and **d** demonstrate acute (white arrow) and chronic (*black arrow*) pulmonary hemorrhage without capillaritis and increased periairway lymphoid tissue (*white asterisk*)

epithelia [14] this is a question that will likely only be addressed through the creation of animal models of Copa syndrome.

Arthritis

Musculoskeletal manifestations are a common feature in patients with Copa syndrome, with approximately 95 % of the patients with physician-diagnosed arthritis. Age of onset is the early teen years, and polyarticular involvement is common among these patients. The most commonly affected joints are the knees and the interphalangeal joints of the hands. Rheumatoid factor is seen in 43 % of reported patients. One patient with chronic polyarthritis was also noted to have elevated level of anti-cyclic citrullinated peptide anitibodies, an autoantibody seen in rheumatoid arthritis that has been correlated with joint destruction [21].

Osteonecrosis was noted in at least 2 patients, one along the femur, patella and tibiofibula; the other patient in bilateral knees that resulted in bilateral knee transplant surgery. It is unclear whether this complication arose due to Copa syndrome or chronic steroid use. One of these patient also developed fatty necrosis (Fig. 3).

Joint pain frequently worsens with pulmonary exacerbations and improves with anti-inflammatory and immunosuppressive therapy. Chronic changes of bony overgrowth, ankylosis or erosive changes were not observed among these patients. Arthritis contributes substantively to disease morbidity, and many patients with Copa syndrome suffer from debilitating joint pain.

Renal Disease

Patients with Copa syndrome also demonstrate increased risk for renal disease. Patients that develop renal disease have an age of onset in their mid to late teen years. In our cohort of 21 affected patients, 44 % had clinical features of glomerular disease marked by either proteinuria and/or decreased renal function. Of those for whom renal biopsy data is available, all had histologic features of glomerulopathy. As opposed to other systemic autoimmune diseases such as SLE or ANCA vasculitis where the patterns of renal injury are distinct, the pathologic features of the glomerular lesions in patients with COPA were disparate: two patients had crescentic disease, one with immune complexes, and one with focal segmental glomerulonephritis without immune deposits; another had IgA nephropathy with necrotizing lesions; and the other had mesangial hypercellularity without immune deposits. While the heterogeneity of the pathologic features of renal disease in these patients does not point to a unifying immunologic mechanisms of renal injury, it is clear that renal involvement can be severe. Necrotizing lesions or cellular crescents were present in up to 75 % of patients with biopsy proven renal disease, and one patient progressed to end-stage renal disease and subsequently underwent renal transplantation despite aggressive immunosuppression. It is not clear at this time if early

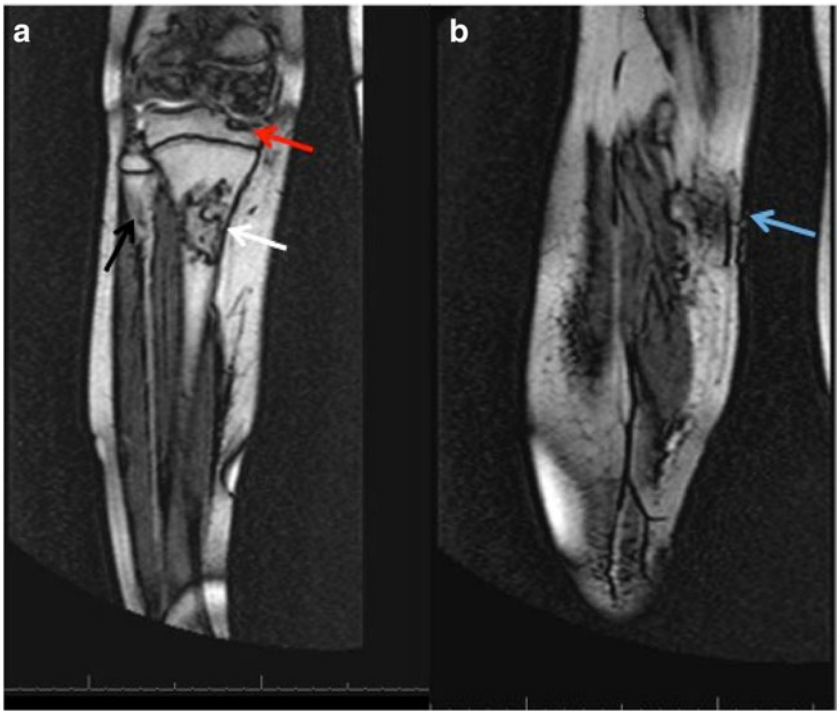

Fig. 3 MRI imaging of arthritis in Copa syndrome. Figure **a** shows an MRI of the distal tibia showing osteonecrosis along the right tibial plateau (*red arrow*), proximal tibia (*white arrow*), and proximal fibula (*black arrow*). Figure **b** shows fatty necrosis along the medial calf, right leg (blue arrow)

recognition of COPA and immunomodulation will reduce or prevent the development of glomerular injury.

Pathogenesis

Linkage to the COPA Gene

Copa syndrome is a hereditary immune dysregulatory primary immunodeficiency resulting from mutations in the *COPA* gene and is listed in the most recent IUIS classification document as such as well as in OMIM (http://www.omim.org/entry/601924) [13, 14]. We identified the molecular cause of the disease through whole exome sequencing and subsequent targeted Sanger sequencing of 5 families consisting of 21 affected members. These genetic studies revealed that all affected members contained single-heterozygous nonsynonymous mutations in the *COPA* gene that result in amino acid changes in the corresponding COPα protein at positions 230, 233, 241, and 243 (K230N, R233H, E241K, D243G). These positions are highly conserved in COPα to level of *Saccharomyces cerevisiae*, and prediction algorithms indicate that they are damaging. When combined the unrelated families provide a LOD score of >5 linking the disease to the *COPA* gene mutations. There is some degree of variable penetrance in Copa syndrome which is not well-understood. Of 16 male patients known to carry *COPA* mutations only 8 had signs of disease whereas 13 of 14 females carrying *COPA* mutations were clinically affected. Thus there may be some gender bias in expressivity and potential for sex chromosome-related modifying factors. This represents an important area for further research in Copa syndrome and will likely reveal important biological mechanisms that might even hold therapeutic potential.

Interestingly, all Copa syndrome causing *COPA* gene variants discovered thus far are restricted to exons 8 and 9, which encode the C-terminal portion of the WD40 domain of the COPα protein (within the 6th of 7 WD40 repeats). It remains unclear how these mutations alter COPα protein structure and whether the single-heterozygous mutations produce disease through a dominant-negative or haploinsufficient effect. That said, what is clear is that the mutations do not alter the total cellular levels of the COPα protein. It is also clear that the *COPA* mutations result in the expression of a mutant COPα protein that is defective in its function.

Potential Pathobiological Mechanisms

Cell homeostasis is essential for maintaining normal cellular function and avoiding undue stresses. In order to maintain proteostasis, the cell uses vesicular carriers to mediate transport and secretion of proteins. Briefly, vesicle formation steps include cargo sorting and coat formation on the donor organelle. Maturation of the vesicle on the donor organelle is then mediated by an activating signal, be it phosphorylation or GTPase activity, resulting in activation of guanine exchange factors (GEFs) leading to the active form of the GTPase Rab/Arf which result in the fission of the vesicle from the organelle membrane. Vesicles then traffic to the acceptor organelle

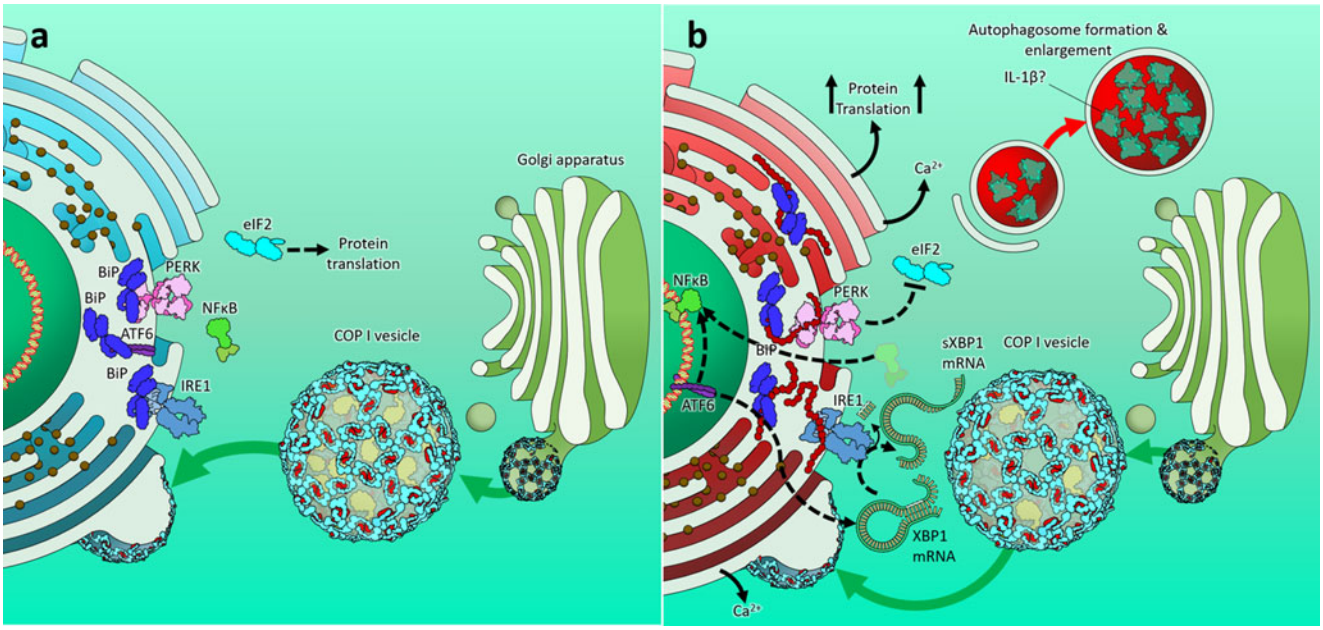

Fig. 4 "Good Cop, bad Cop" the impact of having a mutant COPA and thus defective COPII complex: A working hypothesis. **a**) Within healthy controls, in steady state COPI formation and binding of cargo for retrograde transport occurs on the cis-golgi. The COPI vesicle then migrates toward the ER where fusion occurs with the ER membrane allowing for the protein cargo to be delivered. **b** Within Copa syndrome COPI formation and migration appears to be normal. However, the binding of protein cargo becomes impaired resulting in a deficit of presumably COPII, amongst other machinery responsible for anterograde protein transport. The cell must then compensate for this deficit by increasing new protein translation resulting in an increase in ER stress. This leads to the release of the protein chaperone BiP from the UPR signaling mediators (PERK, ATF6, IRE1) and their activation. This activation of the UPR results in the activation of PERK resulting in inhibition of eIF2 leading to NFκB activation and nuclear translocation. Additionally, the UPR activates ATF6 wich results in increased transcription of XBP1 and further activation of NFκB. Lastly, the UPR activates IRE1 which is responsible for post transcriptional processing of XBP1 transcript. Additionally, be it due to increased ER stress or inappropriate vesicular formation, autophagosomes become excessively large and potentially contribute to increased Inflammasome activation with increased IL-1β processing and secretion

presumably via cytoskeletal motor activity and tether to the accepting organelle utilizing SNAREs. The vesicle then finally fuses to the acceptor organelle membrane and cargo is unloaded. The vesicular pathways responsible for transport between the ER and the Golgi are directed by the protein "coats" or coatomer complexes that associate with the vesicles [22, 23]. Two main coatomers have been identified, one of which is responsible for anterograde vesicular transport, or transport from ER to Golgi, and is known as Coatomer II (COPII) [23]. The other is responsible for retrograde vesicular transport, or transport from the Golgi to the ER, and is referred to as Coatomer I (COPI) [22] (Fig. 4a). COPI is a seven member complex consisting of the proteins encoded by the following genes: *COPA*, *COPB1*, *COPB2*, *COPD*, *COPE*, *COPG*, and *COPZ*. The proteins themselves are referred to by the names of the genes although there are a number of alternates such as COPα for *COPA*, COPβ for *COPB*, and COPβ' for *COPB2*. These seven members form two sub complexes with one known as sub-complex F consisting of COPβ, −δ, −γ, and -ζ, and the second known as sub-complex B consisting of COPα, −β', −and −ε. Sub-complex B is responsible for recruiting protein cargo while sub-complex F is responsible for recruitment of factors responsible for vesicle formation [24]. The sub-complex B members COPα and COPβ' bind protein cargo via dilysine motifs (KKxx, KxKxx) using their WD40 propeller domain [25, 26].

At its cellular basis, Copa syndrome appears to be caused by defective and/or incomplete retrograde transport. Disease-causing *COPA* mutations result in mutant COPα that has a decreased binding capacity for protein cargo (Fig. 4b). This has been demonstrated directly for patient mutations as the normal physiological function of COPα, which is binding dilysine motifs to allow the COPI complex to grab hold of proteins in the golgi, is interrupted. Experimental support for this statement included: 1) the in vitro inability of patient mutant COPα protein to interact and precipitate a dilysine containing "bait" construct; and 2) the inability of a patient derived COPα protein construct to interact with and precipitate a GFP-labeled dilysine reporter protein expressed in a cell. This defect in cargo binding is predicted to lead to a deficit of transport of proteins from the Golgi to the ER. In support of this, patient cells as well as cell lines expressing patient-derived mutant *COPA* demonstrate increased ER stress (as experimentally illustrated by increased BIP and CHOP expression). The effects of the mutations in *COPA* do not lead to abnormal gross COPI localization as demonstrated by standard resolution immunofluorescence microscopy studies and thus the effect leading to ER stress is likely due to a specific

deficit in cargo binding and resulting protein traffic. This increase in ER stress presumably results from a deficit in proteins that are required from the Golgi via recycling back to the ER. The cell must then compensate for this deficit by synthesizing new protein at a rate higher than it would be accustom to as illustrated by the increased ER stress (Fig. 4b).

Whether this hypothesis is correct or not, patient-associated *COPA* mutations do indeed result in an increase in ER stress and the activation of the unfolded protein response (UPR) (Fig. 4b) as demonstrated by increased ATF6 expression in patient cells as well as non-patient cells in which mutant *COPA* is expressed. ER stress and the UPR arises from cellular stresses (nutrient depravation, hypoxia, viral infection) that result in accumulation of unfolded proteins [27, 28]. Increased ER stress leads to direct leakage of calcium from the ER, contributing to activation of proinflammatory transcriptional programs via the activation of NF-κB [29, 30] amongst others.

Additionally the UPR also contributes to inflammation in other ways. The stress sensors PERK, ATF6, and IRE1 are integral parts of the UPR and promote signaling events leading to the production of pro-inflammatory gene products [27, 28, 31]. In steady state, the chaperone protein BiP is associated with the UPR mediators (Fig. 4a). With the onset of ER stress, BiP is sequestered away from the UPR signaling molecules, freeing them to send downstream signals. The three stress sensors then produce different signaling cascades (Fig. 4b). Activation of the first mediator, PERK, results in the phosphorylation and inhibition of elf2α, leading to inhibition of protein translation (Fig. 4b). This inhibition of protein translation via elf2α can result in further NF-κB activation [32]. The second, ATF6, which is responsible for XBP1 and BiP transcription, has also been shown to activate Akt and NF-κB [33] (Fig. 4b). The third, IRE1, is an endoribonuclease and is responsible for the removal of an intron from XBP1 RNA into its active isoform [34] (Fig. 4b). This sXBP1-IRE1α axis is important in B cells – particularly the development of antibody producing plasmablasts [35]. IRE1 has also been shown to interact with TRAF2, resulting in activation of MAPK, JNK, and p38 [36–38]. Altogether IRE1 is involved in activation of TRAF adaptors, MAPKs, and NF-κB, which all mediate inflammation and thus contributing to a multifaceted cellular inflammatory response. This is normally a means of promoting the recognition of cells under stress so that they may be processed or eliminated. In Copa syndrome cells there is an abnormal induction of these pathways owing to the incomplete COPI function and persistent levels of ER stress.

In an attempt to manage induced ER stress, cells turn on the evolutionarily conserved catabolic process of autophagy [39]. During autophagy cytoplasmic contents are engulfed by the autophagosome, which then merges with the lysosome to mediate degradation of the autophagosome and its contents. This represents a physiologic means of containing the cells under stress and even peacefully eradicating them. However, autophagy within cells from Copa syndrome patients is impaired resulting in an increased size and number of autophagosomes with a baseline decrease in the catabolic substrate p62 and over activation of mechanistic target of rapamycin (mTOR). Treatment with torin, a rapamycin homolog, does not enhance autophagosome formation or decrease the catabolic substrate p62 [14]. This might be explained as while torin treatment does decrease mTOR activity it does not reduce it to basal WT levels (unpublished findings LBW and JSO). It is unclear if the aberrant autophagy in Copa syndrome cells represents simply the autophagy process in consistent "overdrive" because of the persistent ER stress, or more directly as a feature of the abnormal vesicular homeostasis [40]. Interestingly, the proteins involved in TMEM173 disease, STING/cGAS, which has phenotypic overlap with COPA syndrome have also been associated with autophagy involved proteins Belcin-1 and ATG1 [41, 42]. Irrespective, this impairment of autophagy in Copa syndrome cells leads one to question the role of autophagy in the pro-inflammatory state of Copa syndrome.

Autophagy has been linked to regulation of the inflammasome through a variety of mechanisms. Some evidence suggests that autophagy can positively regulate the IL-1β response. Since IL-1β does not contain a signal sequence, to direct it to the secretory pathway, it has been shown that IL-1β can be exported in an autophagy dependent manner (Fig. 4b). Specifically by an autophagy regulator (ATG5) dependent mechanism that requires one of the Golgi reassembly stacking proteins (GRASP55 or Rab8a) [43]. Conversely, autophagy has also been associated with negative regulation of the IL-1β response via the degradation of pro-IL1β [44]. How aberrant autophagy relates to the IL-1β response and elevated expression of IL1β mRNA in Copa syndrome cells remains unclear. That said, increased caspase-1 activation has been reported with defects in autophagy mediated by the accumulation of damaged mitochondria, which results in an excessive ROS, and activation of the NALP3 inflammasome by mitochondrial DNA [45, 46], which would presumably increase IL-1β levels. Interestingly, IL-23 production can also be regulated by autophagy in an IL-1β dependent manner, as blocking autophagy allows for enhanced IL-23 transcription through increased IL-1β secretion while activation of autophagy down-regulates IL-23 secretion [47]. Interestingly these two cytokines are associated with priming and maintenance of the Th17 type response that has also been shown to be associated with Copa syndrome. The autoinflammatory components of Copa syndrome are clearly complex and a cause and effect with aberrant autophagy is of interest, but given the increased ER stress caused by the mutation there is much to be proven.

Autophagy itself is also an important process in central selection as thymic epithelia utilize this process in effectively

presenting to and negatively selecting T cells [48, 49]. Thus it is possible that the aberrant autophagy in Copa syndrome itself may be promoting autoreactivity in the T cell compartment – perhaps directly explaining pathological autoantibody production. While that mechanism is purely theoretical at present, the near uniform existence of autoantibodies found in patients is a useful clue. In Copa syndrome, the increase in inflammation from the UPR in conjunction with inflammation from ER stress, and potential contribution of inflammatory cytokines from aberrant autophagy, could explicably produce the perfect environment for autoimmune priming.

Treatment

Currently, Copa syndrome patients are managed in a similar manner to patients who have other pulmonary hemorrhage and autoimmune syndromes. For pulmonary hemorrhages, most patients are treated with either cyclophosphamide or rituximab during exacerbations. Systemic corticosteroids are also often used to manage the acute phases of exacerbations of disease. Additionally, steroids are useful in other autoimmune components such as arthritis, which has been also approached with arthritis-specific disease modifying agents. Remissions in both arthritis and pulmonary disease can be achieved using these medications. However, due to the progressive nature of Copa syndrome several patients have died during acute exacerbations. Maintenance therapies have usually consisted of either methotrexate or azathioprine with intermittent pulses and gradual tapering oral steroids. Other maintenance therapies may include hydroxychloroquine, etanercept, and IVIG at immuno-modulatory dosages. Unlike other pulmonary hemorrhage syndromes, the optimal duration of therapy is not known.

One patient with Copa syndrome has undergone and another has been listed for lung transplantation. The one completed lung transplant was in an adult and was managed according to standard protocols. The patient had near standard post-transplant survival. A single renal transplant was performed in a Copa syndrome patient owing to autoimmune renal failure. This was also managed with standard approaches and the patient still effectively maintains the renal allograft without complications. Hematopoietic stem cell transplant (HSCT) has not, to our knowledge, been performed in a Copa syndrome patient. Given that *COPA* mutations affect both somatic as well as hematopoietic cells (potentially including thymic epithelia) it is unclear as to whether HSCT would be effective in Copa syndrome.

Since Copa syndrome patients have defined defects in cellular mechanisms there fortunately may be an opportunity for more rational therapy based upon targeted modification of the aberrant cell biology. Because defective Golgi-ER transport leads to increased levels of ER stress and ultimately increased mTOR activity, sirolimus, an inhibitor of mTOR, may mitigate the downstream effects of ER stress and may have a role in the treatment of Copa syndrome. Furthermore, because patients with Copa syndrome show an increase in autophagy with abnormal autophagosomes, hydroxychloroquine, which prevents autophagy by inhibiting lysosomal acidification and impairing fusion of the autophagosome with the lysosome, may also be useful for therapy for Copa syndrome. These concepts are currently theoretical and specific testing in vitro is required to understand how these drugs affect the abnormal cellular processes in Copa syndrome before rational use can be recommended for patients.

Conclusion

Copa syndrome is a primary immunodeficiency characterized by immune dysregulation with autoinflammation and autoimmunity. Clinically patients typically have diffuse alveolar hemorrhage, interstitial lung disease and arthritis along with the presence of autoantibiodies. The discovery of the underlying molecular defect in Copa syndrome condition marks the first time that defects in COPI complex proteins have been associated with human disease. Given the autosomal dominant nature of this disease it is anticipated that the condition will not be as rare as many genetically defined primary immunodeficiencies. Ideally better study of the cellular mechanisms underlying Copa syndrome will allow for more rational treatments for those affected on an accelerated timeframe.

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References

1. Picard C, Al-Herz W, Bousfiha A, Casanova J-L, Chatila T, Conley ME, et al. Primary Immunodeficiency Diseases: an Update on the Classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency 2015. J Clin Immunol. Springer US; 2015; 1–31.
2. Melki I, Crow YJ. Novel monogenic diseases causing human autoimmunity. Curr Opin Immunol. 2015;37:1–5.
3. Brown KK. Pulmonary vasculitis. Proc Am Thorac Soc. 2006;3: 48–57.
4. Sacri A-S, Chambaraud T, Ranchin B, Florkin B, Sée H, Decramer S, et al. Clinical characteristics and outcomes of childhood-onset ANCA-associated vasculitis: a French nationwide study. Nephrol Dial Transplant. 2015;30 Suppl 1:i104–12.
5. Siomou E, Tramma D, Bowen C, Milford DV. ANCA-associated glomerulonephritis/systemic vasculitis in childhood: clinical features-outcome. Pediatr Nephrol. 2012;27:1911–20. Springer-Verlag.
6. Morishita K, Li SC, Muscal E, Spalding S, Guzman J, Uribe A, et al. Assessing the performance of the Birmingham Vasculitis Activity Score at diagnosis for children with antineutrophil

cytoplasmic antibody-associated vasculitis in A Registry for Childhood Vasculitis (ARChiVe). J Rheumatol. 2012;39:1088–94.
7. Barile-Fabris L, Hernández-Cabrera MF, Barragan-Garfias JA. Vasculitis in systemic lupus erythematosus. Curr Rheumatol Rep. 2014;16:440–6. Springer US.
8. Kobayashi N, Takezaki S, Kobayashi I, Iwata N, Mori M, Nagai K, et al. Clinical and laboratory features of fatal rapidly progressive interstitial lung disease associated with juvenile dermatomyositis. Rheumatology (Oxford). 2015;54:784–91. Oxford University Press.
9. Burns NS, Stevens AM, Iyer RS. Shrinking lung syndrome complicating pediatric systemic lupus erythematosus. Pediatr Radiol. 2014;44:1318–22. Springer Berlin Heidelberg.
10. Valeur NS, Stevens AM, Ferguson MR, Effmann EL, Iyer RS. Multimodality thoracic imaging of juvenile systemic sclerosis: emphasis on clinical correlation and high-resolution CT of pulmonary fibrosis. AJR Am J Roentgenol. 2015;204:408–22.
11. Liu Y, Jesus AA, Marrero B, Yang D, Ramsey SE, Montealegre Sanchez GA, et al. Activated STING in a vascular and pulmonary syndrome. N Engl J Med. 2014;371:507–18.
12. Jeremiah N, Neven B, Gentili M, Callebaut I, Maschalidi S, Stolzenberg M-C, et al. Inherited STING-activating mutation underlies a familial inflammatory syndrome with lupus-like manifestations. J Clin Invest. 2014;124:5516–20. American Society for Clinical Investigation.
13. Amberger JS, Bocchini CA, Schiettecatte F, Scott AF, Hamosh A. OMIM.org: Online Mendelian Inheritance in Man (OMIM®), an online catalog of human genes and genetic disorders. Nucleic Acids Res. 2015;43:D789–98.
14. Watkin LB, Jessen B, Wiszniewski W, Vece TJ, Jan M, Sha Y, et al. COPA mutations impair ER-Golgi transport and cause hereditary autoimmune-mediated lung disease and arthritis. Nat Genet. 2015;47:654–60.
15. Kuehn HS, Ouyang W, Lo B, Deenick EK, Niemela JE, Avery DT, et al. Immune dysregulation in human subjects with heterozygous germline mutations in CTLA4. Science. 2014;345:1623–7. American Association for the Advancement of Science.
16. Schubert D, Bode C, Kenefeck R, Hou TZ, Wing JB, Kennedy A, et al. Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations. Nat Med. 2014;20:1410–6. Nature Publishing Group.
17. Milner JD, Vogel TP, Forbes L, Ma CA, Stray-Pedersen A, Niemela JE, et al. Early-onset lymphoproliferation and autoimmunity caused by germline STAT3 gain-of-function mutations. Blood. 2015;125:591–9. American Society of Hematology.
18. Lo B, Zhang K, Lu W, Zheng L, Zhang Q, Kanellopoulou C, et al. AUTOIMMUNE DISEASE. Patients with LRBA deficiency show CTLA4 loss and immune dysregulation responsive to abatacept therapy. Science. 2015;349:436–40. American Association for the Advancement of Science.
19. Tashtoush B, Okafor NC, Ramirez JF, Smolley L. Follicular bronchiolitis: a literature review. J Clin Diagn Res. 2015;9:OE01–5.
20. Gomes VCC, Silva MCC, Maia Filho JH, Daltro P, Ramos SG, Brody AS, et al. Diagnostic criteria and follow-up in neuroendocrine cell hyperplasia of infancy: a case series. J Bras Pneumol. 2013;39:569–78.
21. Hamamoto Y, Ito H, Furu M, Hashimoto M, Fujii T, Ishikawa M, et al. Serological and Progression Differences of Joint Destruction in the Wrist and the Feet in Rheumatoid Arthritis - A Cross-Sectional Cohort Study. PLoS ONE. 2015;10:e0136611. Fang D, editor Public Library of Science.
22. Schekman R, Orci L. Coat proteins and vesicle budding. Science. 1996;271:1526–33.
23. Barlowe C, Orci L, Yeung T, Hosobuchi M, Hamamoto S, Salama N, et al. COPII: a membrane coat formed by Sec proteins that drive vesicle budding from the endoplasmic reticulum. Cell. 1994;77:895–907.
24. Waters MG, Serafini T, Rothman JE. "Coatomer": a cytosolic protein complex containing subunits of non-clathrin-coated Golgi transport vesicles. Nature. 1991;349:248–51.
25. Jackson LPL, Lewis MM, Kent HMH, Edeling MAM, Evans PRP, Duden RR, et al. Molecular basis for recognition of dilysine trafficking motifs by COPI. Dev Cell. 2012;23:1255–62.
26. Letourneur F, Gaynor EC, Hennecke S, Démollière C, Duden R, Emr SD, et al. Coatomer is essential for retrieval of dilysine-tagged proteins to the endoplasmic reticulum. Cell. 1994;79:1199–207.
27. Chakrabarti A, Chen AW, Varner JD. A review of the mammalian unfolded protein response. Biotechnol Bioeng. 2011;108:2777–93. Wiley Subscription Services, Inc., A Wiley Company.
28. Diehl JA, Fuchs SY, Koumenis C. The cell biology of the unfolded protein response. Gastroenterology. 2011;141:38–41.e2.
29. Deniaud A, el dein Sharaf O, Maillier E, Poncet D, Kroemer G, Lemaire C, et al. Endoplasmic reticulum stress induces calcium-dependent permeability transition, mitochondrial outer membrane permeabilization and apoptosis. Oncogene. 2008;27:285–99.
30. Pahl HL, Baeuerle PA. Activation of NF-kappa B by ER stress requires both Ca2+ and reactive oxygen intermediates as messengers. FEBS Lett. 1996;392:129–36.
31. Muralidharan S, Mandrekar P. Cellular stress response and innate immune signaling: integrating pathways in host defense and inflammation. J Leukoc Biol. 2013;94:1167–84. Society for Leukocyte Biology.
32. Deng J, Lu PD, Zhang Y, Scheuner D, Kaufman RJ, Sonenberg N, et al. Translational repression mediates activation of nuclear factor kappa B by phosphorylated translation initiation factor 2. Mol Cell Biol. 2004;24:10161–8. American Society for Microbiology.
33. Nakajima S, Hiramatsu N, Hayakawa K, Saito Y, Kato H, Huang T, et al. Selective abrogation of BiP/GRP78 blunts activation of NF-κB through the ATF6 branch of the UPR: involvement of C/EBPβ and mTOR-dependent dephosphorylation of Akt. Mol Cell Biol. 2011;31:1710–8. American Society for Microbiology.
34. Sidrauski C, Walter P. The transmembrane kinase Ire1p is a site-specific endonuclease that initiates mRNA splicing in the unfolded protein response. Cell. 1997;90:1031–9.
35. Iwakoshi NN, Lee A-H, Vallabhajosyula P, Otipoby KL, Rajewsky K, Glimcher LH. Plasma cell differentiation and the unfolded protein response intersect at the transcription factor XBP-1. Nat Immunol. 2003;4:321–9. Nature Publishing Group.
36. Urano F, Wang X, Bertolotti A, Zhang Y, Chung P, Harding HP, et al. Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. Science. 2000;287:664–6.
37. Hu P, Han Z, Couvillon AD, Kaufman RJ, Exton JH. Autocrine tumor necrosis factor alpha links endoplasmic reticulum stress to the membrane death receptor pathway through IRE1alpha-mediated NF-kappaB activation and down-regulation of TRAF2 expression. Mol Cell Biol. 2006;26:3071–84. American Society for Microbiology.
38. Kaneko M, Niinuma Y, Nomura Y. Activation signal of nuclear factor-kappa B in response to endoplasmic reticulum stress is transduced via IRE1 and tumor necrosis factor receptor-associated factor 2. Biol Pharm Bull. 2003;26:931–5.
39. Yorimitsu T, Nair U, Yang Z, Klionsky DJ. Endoplasmic reticulum stress triggers autophagy. J Biol Chem. 2006;281:30299–304. American Society for Biochemistry and Molecular Biology.
40. Claerhout SS, Dutta BB, Bossuyt WW, Zhang FF, Nguyen-Charles CC, Dennison JBJ, et al. Abortive autophagy induces endoplasmic reticulum stress and cell death in cancer cells. PLoS ONE. 2012;7,e39400. Langsley G, editor.
41. Liang Q, Seo GJ, Choi YJ, Kwak M-J, Ge J, Rodgers MA, et al. Crosstalk between the cGAS DNA sensor and Beclin-1 autophagy

protein shapes innate antimicrobial immune responses. Cell Host Microbes. 2014;15:228–38.
42. Konno H, Konno K, Barber GN. Cyclic dinucleotides trigger ULK1 (ATG1) phosphorylation of STING to prevent sustained innate immune signaling. Cell. 2013;155:688–98.
43. Dupont N, Jiang S, Pilli M, Ornatowski W, Bhattacharya D, Deretic V. Autophagy-based unconventional secretory pathway for extracellular delivery of IL-1β. EMBO J. 2011;30:4701–11. EMBO Press.
44. Harris J, Hartman M, Roche C, Zeng SG, O'Shea A, Sharp FA, et al. Autophagy controls IL-1beta secretion by targeting pro-IL-1beta for degradation. J Biol Chem. 2011;286:9587–97. American Society for Biochemistry and Molecular Biology.
45. Nakahira K, Haspel JA, Rathinam VAK, Lee S-J, Dolinay T, Lam HC, et al. Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. Nat Immunol. 2011;12:222–30.
46. Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. Nature. 2011;469:221–5.
47. de Castro Peral C, de Castro CP, Jones SA, Ní Cheallaigh C, Cheallaigh CN, Hearnden CA, et al. Autophagy regulates IL-23 secretion and innate T cell responses through effects on IL-1 secretion. J Immunol. 2012;189:4144–53. American Association of Immunologists.
48. Schuster C, Gerold KD, Schober K, Probst L, Boerner K, Kim M-J, et al. The autoimmunity-associated gene CLEC16A modulates thymic epithelial cell autophagy and alters T cell selection. Immunity. 2015;42:942–52.
49. Nedjic J, Aichinger M, Emmerich J, Mizushima N, Klein L. Autophagy in thymic epithelium shapes the T-cell repertoire and is essential for tolerance. Nature. 2008;455:396–400.