



HAL
open science

Sarcoidosis and the mTOR, Rac1, and Autophagy Triad

Yves Pacheco, Clarice X. Lim, Thomas Weichhart, Dominique Valeyre,
Abderrazzak Bentaher, Alain Calender

► **To cite this version:**

Yves Pacheco, Clarice X. Lim, Thomas Weichhart, Dominique Valeyre, Abderrazzak Bentaher, et al.. Sarcoidosis and the mTOR, Rac1, and Autophagy Triad. Trends in Immunology, 2020, 41, pp.286 - 299. 10.1016/j.it.2020.01.007 . hal-03489655

HAL Id: hal-03489655

<https://hal.science/hal-03489655v1>

Submitted on 22 Aug 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

OPINION

SARCOIDOSIS and the “mTOR, Rac1, and autophagy” triad

Pacheco Y¹, Lim C², Weichhart T², Valeyre D³, Bentaher A¹ and Calender A^{1,4,*}

- 1 Inflammation and Immunity of the Respiratory Epithelium, Lyon 1 Claude Bernard University, EA-7426 (PI3), Pierre-Bénite, France
- 2 Center for Pathobiochemistry and Genetics, Institute of Medical Genetics, Medical University of Vienna, Vienna, Austria
- 3 Department of Pulmonology, Avicenne Hospital, AP-HP, EA-2363, University Paris 13, Bobigny, France
- 4 Department of Molecular and Medical genetics, Hospices Civils de Lyon, University Hospital, Bron, France

*Correspondence:

alain.calender@chu-lyon.fr

(A. Calender)

ABSTRACT

Sarcoidosis is an enigmatic multisystem disease characterized by the development and accumulation of granulomas: a compact collection of macrophages differentiated into epithelioid cells, which are associated with helper Th1 and Th17 cells. Although no single causative factor has been elucidated to understand the occurrence of sarcoidosis in humans, its etiology has been related to microbial, environmental, as well as genetic factors. Here, we examine how these factors play a role in sarcoidosis pathogenesis; we propose that a dysfunction in mTOR, Rac1, and autophagy-related pathways could hamper pathogen or non-organic particle clearance, as well as participate in T-cell and macrophage dysfunction, driving granuloma formation. This concept opens new avenues for potentially treating sarcoidosis and may serve as blueprint for other granulomatous disorders.

KEY WORDS

Sarcoidosis, granuloma, genetics, Whole Exome Sequencing, autophagy, mTOR, Rac1, Azathioprine, Rapamycin, monocyte, T cell, Treg, Multinucleated Giant Cell

IS THERE A ROLE FOR AUTOPHAGY IN SARCOIDOSIS AND GRANULOMATOUS DISEASES?

Sarcoidosis in mammals is characterized by the accumulation of granulomas, a compact collection of lymphocytes and macrophages that have differentiated into epithelioid cells (**EC**), and that sometimes fuse to form multinucleated giant cells (MGC's) [1]. The mechanism of granuloma formation remains unknown but the contribution of an aberrant immune response to environmental triggers has been implicated [2]. Granulomas are usually formed in response to external triggers, such as parasites (e.g. *Leishmania major*) or foreign bodies, to eradicate the intruding agent. Moreover, granulomas are thought to limit the spread of pathogenic bacteria such as *Mycobacterium tuberculosis* (MBT) [3,4]. In sarcoidosis, various infectious and non-infectious agents have been observed in granuloma cores, such as *Propionibacterium acnes* (PA) or serum amyloid A (SAA) deposits [5]. Moreover, defective mechanisms driving the elimination of microbial-derived particles, mainly autophagy [**BOX 1**], have been studied in Crohn's disease (CD) -- a granulomatous disease affecting the digestive tract (inflammatory bowel disease) [6-9]. Indeed, germline mutations in genes encoding autophagy-related proteins, such as NOD2 (Nucleotide-binding and oligomerization-domain containing 2), ATG16 (autophagy related protein 16) and IRGM (Immunity Related GTPase Family) have been found in familial forms of CD [10-12]. In addition, gene-expression array analysis performed in mycobacterial leprosy, a granulomatous disease, has shown significant upregulation of *ATG* genes in anti-inflammatory T lymphocytes [13]. Recently, Whole Exome Sequencing (WES)

analysis of familial forms of sarcoidosis suggested that genetic predisposition to the disease might be related to a cluster of germline mutations in genes encoding various regulators of mTOR (mammalian Target Of Rapamycin) and autophagy-related proteins [14,15] [BOX 2]. Accordingly, mTOR signaling has been previously implicated in sarcoidosis pathogenesis. For instance, in one study, spontaneous formation of **non-caseating granulomas**, as well as restoration of normal tissue homeostasis following mTOR inhibitor (rapamycin) treatment, was reported in a Tsc2-defective (*Tsc2*^{-/-} knockout) mouse model of constitutive mTORC1 activation in macrophages [16]. In another study, RNA-seq data revealed mTORC1 (mTOR related Complex 1) signaling, in CD68 macrophages from a cutaneous sarcoidosis patient who had exhibited clinical and histological remission of the disease following treatment with the **JAK** (Janus Kinase 1) inhibitor Tofacitinib (which activates autophagy) [17]. Another patient who developed de novo systemic sarcoidosis also recovered, and exhibited normal chest imaging and no granuloma-like lesions following administration of sirolimus (rapamycin), an mTOR inhibitor [18]. As mTORC1 is a negative regulator of mTOR [19] (BOXES 1 and 2), the contribution of aberrant autophagy processes and degradation of cargo (i.e. microbial products, protein aggregates and damaged mitochondria) during sarcoidosis granuloma formation remains to be elucidated [BOXES 1 and 2].

Here, we posit a multifactorial disease etiology hypothesis where environmental triggers, and dysfunctional autophagy due to genetic mutations affecting mTOR and Rac1 pathways, contribute to promoting sarcoidosis pathogenesis [Key Figure, Figure 1].

GENETIC SUSCEPTIBILITY in SARCOIDOSIS

The existence of familial forms of sarcoidosis suggests that genetic predisposition plays an important contributory role in sarcoidosis pathogenesis in humans. A recent case-control-family retrospective study nested in Swedish population-based registers (Swedish Research Council) showed that having a relative with sarcoidosis was a risk factor, and estimated the mean heritability of sarcoidosis at 39% [20]. Despite many years of genetic association studies on large cohort of sarcoidosis patients, no major genes have been implicated with a significant odd-ratio risk in predisposition [21]. To overcome the issue of **missing heritability**, next-generation DNA sequencing techniques [BOX 3] comparing affected family members versus unaffected familial internal controls have been recently described to analyze whole exome sequencing (WES) in familial forms of the disease [14,22]. The subtraction of genotypes between an affected child and his/her parents provided a series of recessive and de novo variants in genes related to i) autophagy and intracellular trafficking (e.g. *Sec16A*, *AP5B1*) ii) G-proteins regulation (e.g. *OBSCN*, *CTNND2*, *ADGRV1*), iii) T-cell activation (e.g.

IDO2, IGSF3) and iv) cell cycle and immunological synapses (e.g. *SPICE1, KNL1*) [14]. Recently, WES screening of 5 families sharing at least two first-degree affected relatives compared the genotypes of patients to intrafamilial healthy controls [22]. Analysis of exome data allowed to highlight mTOR signaling and autophagy as the top pathways enriched amongst the transcripts affected by the selected variants. Part of the results overlapped with WES data in German families, highlighting enrichment in pathways related to cell survival and migration, calcium metabolism, as well as cell adhesion processes [23].

Rac1 (Ras-related C3 botulinum toxin substrate 1) is an important regulator of autophagy and has also been associated with sarcoidosis (15). Rac1 is a ubiquitously expressed member of the Rho (Ras homologous) GTPase family that plays important role in cytoskeletal reorganization, gene expression, cell morphology, cell motility, activation of the NADPH (Di hydro nicotinamide-adenine dinucleotide phosphate) oxidase complex; it is regulated by GTPase Activating Protein (GAP) and Guanine nucleotide exchange factors (GEFs) [24]. Of note, Rac1 is one of the main targets of the immunosuppressive compound azathioprine (AZT), used in corticosteroid-resistant forms of sarcoidosis and in CD treatment [25] [BOX 4]. In one of the studies segregating affected sarcoidosis cases and healthy relatives, genomic subtraction of genotypes identified three rare and deleterious mutations, one in the gene encoding the IL-17A (interleukin-17-A) receptor, and the two others in main genes encoding Rac1, EPHA2 (Ephrin Receptor EphA2) and KALRN (Kalirin) [15]. The activation of Rac1 via EPHA2 and KALRN occurs downstream of various Pathogen Recognition Receptors (PRRs) such as NOD2 and TLR4 and TLR9 Toll-like receptors (TLRs) [26,27]. TLRs play a critical role in innate immunity by detecting invading pathogens and promoting autophagy [28,29]. Accordingly, polymorphisms in different TLRs have been linked to sarcoidosis disease progression [30]. Specifically, *NOD2*, the first CD susceptibility gene to be discovered, belongs to a class of cytoplasmic PRRs and responds to pathogen (bacteria and virus) associated molecular patterns (PAMPs) to activate NFκB (Nuclear factor kappa B) and MAPK (mitogen-activated protein kinase) pathways [31] [Figure 2]. *NOD2* and *NOD1* recruit the autophagy protein ATG16L1 to the plasma membrane at the bacterial entry site, and contribute to autophagosome engulfing of invading pathogens [32].

In addition, many polymorphisms expanding throughout the extended *HLA* major histocompatibility complex (MHC) region -- either in class I or II -- have been described in sarcoidosis [1]. Among them, polymorphisms occur in *BTNL2* (Butyrophilin-like 2), a MHC class II associated transmembrane glycoprotein that acts as a negative regulator of T cell proliferation, and which shares a splicing variant (rs2076530), commonly observed in control populations (allele frequency: 41,9%) but more frequently in sarcoidosis patients with an odd-ratio of 2.0 (95% CI: [1.32-3.09]) [33,34]. *BTNL2* can induce the expression of *FOXP3* factor (forkhead box P3) – a key transcription factor responsible for

the formation of regulatory T-cells [35]. In these cells, BTNL2 acts by inhibiting serine-threonine kinase 1 (AKT) activity, with a putative impact on mTOR activity and therefore, autophagy regulation [36] [Figure 2]. Taken together, sarcoidosis patients can harbor various non-related single-nucleotide polymorphism (SNP) variants affecting innate activation of monocytes/macrophages and subsequent autophagy induction.

INFECTIOUS and NON INFECTIOUS TRIGGERS OF SARCOIDOSIS

Other than genetic abnormalities, different triggers have been implicated in granuloma induction in sarcoidosis and the disease develops probably in genetically predisposed individuals with exposure to an as-yet unknown antigen [37]. Currently, defining the role of infectious agents in sarcoidosis remains a major question. Are these causal factors, or are they themselves the result of a defect in intracellular clearance? A large retrospective meta-analysis of sarcoidosis patients reported a significant association between *Propionibacterium acnes* (PA), mycobacteria (MB) and sarcoidosis [5]. Evidence of PA bacteria in human sarcoidosis granulomas was reported [38]. Experimental pulmonary granulomas mimicking sarcoidosis have been induced in a mouse model of sensitization with heat-killed PA and complete Freund's adjuvant after repeated injections in the footpads of mice [39]. Mouse RAW-264.7 macrophages cell lines, infected by *P. acnes* were shown to increase autophagy, largely killing bacteria through this process [40]. Furthermore, analysis of sarcoidosis-associated lung microbiota in sarcoidosis patients revealed enrichment of two anaerobic bacterial species, *Atopobium* (Gram+) and *Fusobacterium* (Gram-) bacteria, compared to healthy individuals [41]. *Fusobacterium* targets TLR4 and MYD88 (myeloid differentiation primary response protein) induced innate immune signaling to activate autophagy in human colorectal cancer cells [42]. Similarly, MBT infection can cause granulomatous disease, but usually leads to granulomas with central 'caseous necrosis', which is absent in sarcoidosis [2]. MBT is an intracellular pathogen that inhibits accumulation of autophagic vacuoles in macrophages [43]. Recent experiments in a mouse model of MBT-infected bone marrow macrophages suggested that the molecular interactions linking MBT to autophagy, implicated a process called ubiquitin-mediated **xenophagy** [44]. In this model, further studies have shown physical interactions between ubiquitin 1 and MBT surface proteins such as Rv1468c, which contain a eukaryotic-like ubiquitin-associated (UBA) domain [44,45].

Among non-infectious particles, amyloid deposits of Serum Amyloid A (SAA) have been detected in various human lung granulomatous disorders, such a tuberculosis and non-tuberculosis mycobacteria infection, leprosy and sarcoidosis [46]. Indeed, SAA has been proposed to contribute to persistent immune responses in sarcoidosis by activating TLR2 in alveolar macrophages and transformed human

cell lines (such as HEK-293) [47]. Of note, SAA induces nitric oxide production and activates autophagy, as shown in mouse RAW-264.7 macrophages cell lines [48].

From another angle, experimental mouse models have provided evidence of lung granulomatous inflammation after inhalation of MWCNT (Multi Wall Carbon NanoTubes) nanoparticles (NNP) [49,50]. Regarding NNPs, a link between NNP and sarcoidosis was also suggested during the NYC World Trade Center disaster in September 2001, identifying a significant increase in "sarcoidosis-like" granulomatous pulmonary disease among New York Fire Department rescue workers compared to the basal incidence of the disease in non-exposed individuals [51]. Of relevance, autophagy can be induced by NNP (MWCNT, amorphous silica or copper oxide) in mouse models and human cell lines such as Human Umbilical Vein Endothelial Cells (HUVEC) [52]. Transmission Electron Microscopy analysis of these NNP-charged cells has shown an impairment of autophagic flux related to accumulation of NNP in autophagosomes and lysosomes, with the accumulation of undegraded autophagosomes[53]. Along these lines, experimental inclusion of 30 nm polystyrene NNP in RAW-264.7 murine macrophages blocked vesicle transport in the endocytic system as well as the distributions required for cytokinesis, resulting in bi-nucleated cells, an interesting observation which might be compared to the multinuclear aspect of MGCs in sarcoidosis [53].

Collectively, current evidence suggests that there is no single environmental trigger in sarcoidosis, but all infectious and/or non-infectious triggers described so far are potent inducers of autophagy [Key Figure, Figure 1]. Genetic defects in autophagy-related genes might be a potent factor for deficient clearance of these triggers, thus resulting in granuloma formation.

FROM MACROPHAGES to GRANULOMAS: WHAT IS THE ROLE OF AUTOPHAGY IN SARCOIDOSIS?

A remarkable feature of sarcoidosis is the compartmentalization of macrophages in the affected organs to initiate the formation and maintenance of granulomas [2]. Among PRRs, nucleotide-binding domain, leucine-rich repeat containing family proteins (NLRs) and TLRs play a critical role in innate immunity by detecting invading pathogens and interacting with the molecular pathways of autophagy [54-56]. In CD patients carrying mutations in NOD2 and/or ATG16L1, monocyte-derived macrophages are unable to restrict the replication of invasive *Escherichia coli* and release significant amounts of interleukin IL-6 and TNF- α (Tumor Necrosis Factor alpha) [6] [Figure 3]. Similar to CD, a role for TLRs and NLRs has been suggested by the observation that human blood monocytes express high amounts of TLR2 and produce large amounts of TNF- β , IL-1 β and IL-6 when stimulated with TLR2 ligands relative to unstimulated cells. [57]. In a TLR2 knockout (*Tlr2*^{-/-}) mouse model, PA-induced

granulomatous lung inflammation was significantly reduced compared to wild type (WT) C75BL/6 animals, suggesting a role for TLR2 in immune activation and granuloma formation in mice [58]. Indeed, a genetic linkage between *TLR2* gene polymorphisms and the risk of sarcoidosis has been previously established [59].

The first steps of granuloma formation typically involve the aggregation of cells as seen by the observation that treatment of peripheral blood mononuclear cells (PBMC) isolated from sarcoidosis patients with purified protein derivative (PPD)-coated polystyrene beads results in the formation of granuloma-like multicellular aggregates [60] [Figure 3]. This recently developed in vitro human sarcoidosis granuloma model has allowed the identification of an IL-13-regulated **M2 macrophage polarization** pathway, which may predispose to granuloma formation [61] [Key Figure, Figure 1]. Indeed, anti-inflammatory M2 macrophage polarization occurs in sarcoidosis granulomas [62]. In the mTORC1-dependent mouse granuloma model, macrophages have been similarly polarized to an M2 profile [16]. Among signaling networks, STAT6 (Signal Transducer and Activator of Transcription 6) plays a pivotal role in transducing IL-4 and IL-13 cytokine signals, both of which promote macrophage cell-cell fusion [63]. This mechanism implicates the receptor-coupled intracellular JAK signaling network, an interesting functional link, because JAK inhibitor (Tofacitinib or Ruxolitinib) therapy was recently demonstrated to be efficient for resolution of cutaneous sarcoidosis in a JAK2 mutation-positive patient [17,64]. Indeed, IL-13 activates autophagy in some models, such as airway epithelial cells in ATG16L1 knockout (*Atg16l1*^{-/-}) mice compared to WT controls. [65].

Inflammation-associated factors that stimulate macrophages have also been linked to sarcoidosis pathogenesis [Figure 2]. For instance, monocytes of sarcoidosis patients show an enhanced ability to aggregate in vitro after stimulation with TNF- β and interferon- γ , a process which has been associated to ICAM-1 (Intercellular Adhesion Molecule 1) induction of in human alveolar macrophages (AM) [66]. Other cytokines, such as CCL20 (Chemokine CC motif Ligand 20) produced by AM in sarcoidosis, induce the aggregation of monocyte-derived dendritic cells (MoDCs) that further fuse to form MGCs upon exposure to IL-17A [2]. In a mouse model of granuloma induced by *Paracoccidioides brasiliensis*, increased amounts of Th17-associated cytokines (IL-6, IL-23, IL-17A) were observed relative to controls (67). Moreover, IL-17 was shown to promote M2 macrophage differentiation in an IL-17 knockout (*Il17*^{-/-}) mouse model [68] [Figure 3].

In summary, in sarcoidosis patients, M2 macrophage polarization and further cell aggregation can involve PRRs such as TLR2, various cytokines such as IL-13, IL17-A, CCL20 and adhesion molecules (e.g. ICAM1), closely linked to biochemical pathways including mTOR and STAT6, which can interfere with autophagy. Genetic alterations in autophagy-related pathways can in turn lead to ineffective

clearance of the environmental triggers (such as bacteria, viruses and inorganic particles including NNPs) and subsequently, initiate granuloma formation.

AUTOPHAGY AND T-CELL FUNCTION IN SARCOIDOSIS

The appearance of helper Th1/Th17 lymphocytes in sarcoidosis is well documented [75]. A compartmentalization of CD4⁺ Th1 has been observed in lungs and other affected organs. Th17 effector T cells were evaluated in human BAL (bronchoalveolar lavage) cells by flow cytometry and shown to contribute to alveolitis/granuloma formation and putatively, to the fibrotic process that has been observed in some patients, under the control of cytokines such as CCL20 [69].

Several studies have suggested that activation of human alveolar T cells from sarcoidosis patients occurs, with a clustering of these T-cells during the S/G2+M cell cycle phase, in addition to their oligoclonality [70,71]. Indeed, T cell clones established from the BAL of sarcoidosis patients produce high amounts of IL-1 β , IL-10, TNF- β and GM-CSF [72].

Recent arguments have been proposed that link autoimmunity to sarcoidosis [73]; first, an oligoclonal quota of antigen-specific AV2S3 -- a particular subset of alveolar T lymphocytes identified in patients with an HLA-DRB1-0301 haplotype -- can recognize inherent peptides such as vimentin [74,75]; secondly, a Th1/Th17-driven inflammatory process has been observed in sarcoidosis that seems to be identical to that seen in certain auto-immune diseases such as rheumatoid arthritis and multiple sclerosis [76].

In the context of autoimmune disorders or MBT infection, recent investigations suggest that Th17 cells might be implicated at all stages of granuloma evolution -- from the initial steps of fusion, to further maintenance, and in some cases, resolution [77,78]. Survival and proliferation of Th17 effector CD4⁺ T cells are IL-23 dependent as shown *ex vivo*, in a model of human autoimmune arthritis [79]. This is of interest as both IL-17 and IL-23 are two markers of inflammation during the course of human sarcoidosis [80] **[Figure 3]**.

Indeed, increased expression of IL-17A has been described in patients with sarcoidosis relative to controls [81]. IL-17A positive CD4⁺ T cells have also been shown to infiltrate sarcoid lung lesions from patients at different stages of disease evolution [69]. A recent case report suggested the efficiency and safety of anti-IL17-A monoclonal antibody (secukinumab) treatment in a patient who had developed certolizumab (TNF- α inhibitor)-induced sarcoidosis [82]. Evidently, further testing will be required.

Indeed, the precise mechanisms of IL-17A signaling in autophagy regulation remain unknown and discordant data have been reported on this functional link. In fact, IL-17A stimulates autophagy in MBT-infected monocytes from patients with active tuberculosis and more specifically in severe forms of this infectious disease [78]. Conversely, a novel IL-17A-mediated signaling pathway has been shown to inhibit autophagy through activation of PI3KCA (Phosphoinositide-3-kinase catalytic alpha), a key factor involved in cell growth, survival, proliferation, motility and morphology [83]. JAK/STAT signaling may have an essential mediating role by transducing signals from IL-17 receptors, as shown in human U-937 monocytic leukemia cells [84]. The JAK/STAT pathway inhibits autophagy, and STAT1 expression is increased in human sarcoid tissues relative to controls [17]. Autophagy can influence Th17 cell differentiation and more generally, it plays a role in T-cell homeostasis through the regulation of calcium and nutrient metabolism [85]. Therefore, deleterious mutations in autophagy-related genes and mTOR signaling pathways could impact T lymphocyte homeostasis and expansion [Figure 3].

Sarcoidosis patients are characterized by exhibiting negative tuberculin skin-tests, suggesting a T-cell hypo-responsive state which might be related to an intrinsic defect in CD4⁺ and FoxP3⁺ regulatory T cells (Treg) [86]. In a *Tsc1* knockout (*Tsc1*^{-/-}) mouse model, mTOR signaling has been deemed to play a critical role in dictating the outcome of Treg and Th17 cell proliferation and maturation; loss of *Tsc1*, a negative regulator of the mTORC1 complex, enhances mTOR activity which appears downstream of the IL-2R pathway, as a major regulator of anergy versus activation [87]. In a mouse model of ovalbumin-induced intestinal inflammation, mTOR regulated autophagy and FoxP3⁺ Treg cells impaired the autophagic machinery of dendritic cells in a CTLA-4 (cytotoxic T-lymphocyte associated protein 4)-dependent manner [88]. This is relevant, as CTLA-4 is a negative regulator of T-cell activation expressed on activated T-cells, and exon 1 polymorphisms have been associated with sarcoidosis susceptibility [89]. Moreover, anti-CTLA4 monoclonal antibody (Ipilimumab) treatment in melanoma patients can induce cutaneous and bronchial granulomas, mimicking sarcoidosis [90].

Of clinical relevance, the occurrence of sarcoid lesions during immune checkpoint blockade treatment by anti-PD-1/PD-L1 (Programmed cell Death ligand 1) antibodies (Nivolumab) in various human cancers such as melanoma and lung adenocarcinoma has been documented [91]. In a murine model of fatal visceral Leishmaniasis, PD-1/PDL-1 blockade inhibited autophagy in CD4⁺ and CD8⁺ T cells [92]. In patients presenting thoracic and cutaneous sarcoid-like reactions associated with anti-PD1 therapy for cancer, a rapid decrease of PD-L1 expression preceded the spontaneous resolution of sarcoid-like reactions [91]. Taken together, it is reasonable to speculate that defective autophagy in T-cells due to PD-1/PD-L1 signaling blockade might play a putative role in eliciting sarcoidosis by

controlling T-cell activity and proliferation [Figure 3]. However, robust testing is evidently warranted to analyze this hypothesis.

Autophagy and its regulation by mTOR play a crucial role in T cell fate. Genetic abnormalities in these pathways might help explain imbalances between Treg and Th17 functions. We posit that this crosstalk might contribute to promoting Th17-mediated inflammation in sarcoidosis patients.

OTHER SIGNAL TRANSDUCTION PATHWAYS INFLUENCING AUTOPHAGY IN SARCOIDOSIS

Aside from the previously detailed pro-inflammatory pathways, other processes of clinical relevance in sarcoidosis, have been implicated in mediating T cell disorders, macrophage activation and granuloma formation. These include NF κ B (Nuclear factor kappa B)[93], IFN γ /STAT1 [94], G α i (Gi protein alpha subunit) [95], RIP2/IRAK (receptor interacting protein 2, Interleukin-1 receptor-associated kinase 1) [96], p38 MAP kinase (p38 mitogen-activated protein kinase) and MKP1 (Mitogen-Activated Protein Kinase Phosphatase 1) [97], some of them being closely related to autophagy pathways [Figure 2].

The immune response in sarcoidosis is mediated by type I inflammatory cytokine pathways such as TNF α , IL1 β , IL-6, promoted by NF κ B [93], and by CXCL9, CXCL10 or CXCL11, promoted by STAT1 dependent signaling [94]. Moreover, STAT1 signaling plays a critical role in modulating the polarization of T helper cells [98].

MicroRNA gene expression analysis in PBMC from sarcoidosis patients has shown activation of the JAK/STAT pathway [99]. JAK/STAT inhibits the expression of Unc-51-like kinase (ULK-1) and autophagosome formation in human fibrosarcoma cells [100]. Increased expression of MAP kinase 4, IRAK1, and RIP2 have also been observed in AM of sarcoidosis patients relative to cells obtained from control individuals [96]. Enhanced activation of the p38 MAPK pathway occurs in BAL cells from sarcoidosis patients in response to TLR or NLR stimulation, and has been linked to a failure in the expression of MKP-1 (MAP kinase phosphatase 1) compared to control individuals [97]. This last model suggests that in sarcoidosis, MKP-1 deficiency might lead to overexpression of STAT1 and thus, inhibition of autophagy. Inhibition of JAK by Tofacitinib has been successful in treating cutaneous sarcoidosis and this might be explained in part by downregulation of STAT1 in treated patients, although this remains to be further assessed [14].

In the intestinal environment, chronic NOD2 stimulation in human macrophages induces upregulation of NF- κ B and a significant decrease of cytokine expression, such as TNF α , IL-6, IL-8, IL-

10, relative to non-stimulated cells. [31]. As previously mentioned, this regulation can be impaired in CD patients harboring NOD2 mutations, and potentially, in sarcoidosis patients bearing an NOD2 Gly908Arg missense variant [15], which might account for some of the biochemical abnormalities observed.

A defect in the negative regulation of adenylate cyclase mediated by the inhibitory G-protein G α i was also observed in human sarcoidosis peripheral blood purified T cells, which might help explain the low proliferative response of lymphocytes to mitogenic lectins during this disease [101]. Activation of CXCR4 signaling in lymphoid cells increases autophagic activity by stimulation of the autophagic regulator LC3 (microtubule-associated protein light chain 3) through a G α i-RAS (rat sarcoma)-VAV oncogene-rac1 pathway [101] [Figure 2].

Given the presumed importance of IL-17 in sarcoidosis, understanding this pathway of regulation is important. Accordingly, Rac1 Rho GTPase regulates *IL17A* transcription by binding to the *IL17A* promoter in a complex with ROR γ t (Retinoic Acid Receptor related Orphan nuclear receptor gamma), -- a crucial factor for pro-inflammatory Th17 cell differentiation [102]. In this study, *Tiam1*^{-/-} knockout mice were used to demonstrate the regulation of Rac1; with Tiam1, acting as a GEF. We hypothesize that genetic mutations observed in some regulatory factors of Rac1 might potentially disturb the expression of *IL17A*, favoring a pro-inflammatory state that might promote sarcoidosis [15].

T-cell antigen receptor (TCR), CD28 and CXCR4 interact within immunological synapses -- the interface between an antigen-presenting cell or target cell, and a lymphocyte [103] [Figure 2]. CD4⁺CD28⁺ T cells are expanded in BAL cells from sarcoidosis patients [104]. Upon ligation, the TCR associates with and activates CXCR4 in order to activate PREX1 (Phosphatidylinositol 3,4,5-trisphosphate-dependent Rac-exchanger 1 protein) to stabilize cytokine expression [105]. Rac1 appears to bind directly to mTOR in response to growth-factor stimulation and regulates its activity by mediating mTORC1 and mTORC2 localization at specific membranes [106]. Autophagy potently inactivates Rac1 and conversely, active Rac1 competes with LC3, which might in turn, prevent formation of autophagosomes [107].

Taken together, several biochemical pathways have been shown to be disturbed in human sarcoidosis. Many of them interact with autophagy. Pharmacological approaches to target some of these pathways are currently in development [BOX 4].

CONCLUDING REMARKS

Current research has suggested that both environmental and genetic factors play a role in sarcoidosis. We hypothesize that genetic mutations observed in this multifactorial disease mostly occur on autophagy-related genes and two regulatory hubs, mTOR and Rac1, which can hamper the clearance of pathogens (opportunistic viruses or bacteria) or non-organic particles, and alter both macrophages and T cell responses. In agreement with this hypothesis, different authors have reported the presence of bacteria, viral nucleic acids, endogenous amyloid aggregates and/or NNP in sarcoidosis granulomas and have assessed autophagy as a stress-response and quality control pathway. We posit that Rac1 plays a spatiotemporal role, and mTOR, a fundamental nutritional role during intracellular vesicle trafficking; these pathways may modify autophagy in sarcoidosis. Consistent with this theory, some studies suggest that multifactorial diseases such as CD might be determined by a susceptibility-gene interaction in combination with additional environmental factors and commensal bacteria. This might add another layer of complexity to partly explain why the disease occurs in only a small proportion of individuals carrying common risk alleles of disease susceptibility genes. However, many questions remain unanswered (see outstanding questions). We clearly need functional studies focusing on sarcoidosis patients' macrophage and T cell biochemical disturbances (both blood and alveolar), and to exploit the experimental granuloma models established to date.

Acknowledgements

We thank the patients for their kind participation and continued interest in this study. This work was performed in the frame of a national clinical and research group working on sarcoidosis, Group Sarcoidosis France (GSF) and supported by grants from "Fondation Maladies Rares" (FMR – France), and French Research Minister INNOVARC-DGOS ref 12-027-0309. T.W. is supported by grants from the Austrian Science Fund (FWF) P27701-B20 and P30857-B28, by the Vienna Science and Technology Fund (WWTF) LS18-058, and by the Foundation for Sarcoidosis Research.

LEGENDS TO FIGURES

Key Figure, Figure 1. Autophagy and granuloma crosstalk. The diagram illustrates the interplay between autophagy and granuloma formation in human diseases and in experimental animal models, using various external triggers. Natural triggers can saturate the endolysosomal system with the formation of granulomas, especially in patients with functional abnormalities in the autophagy process. The experimental models are described in the text and highlight the potential physiological pathways involved, such as mTOR signaling.

Abbreviations: SAA: Serum Amyloid Protein A; mTOR: Mechanistic Target of Rapamycin; Rac1: Ras Related Botulinum Toxin Substrate 1; S6K1: Ribosomal Protein S6 Kinase; JAK: Janus Kinase; STAT1: Signal Transducer and Activator of Transcription 1; TSC2/KO: Tuberin or Tuberous Sclerosis 2 knock-out mouse model; IL-13: interleukin 13;

Figure 2. Biochemical pathways involved in sarcoidosis. For each of the functional networks, depicted are: the triggers, the cytokines and their receptors, and the main metabolic axes involved in mechanisms leading to inflammation and regulation of autophagy. As stated in the main text, whole exome studies of families predisposed to sarcoidosis followed by gene-enrichment screening have identified pathogenic constitutional mutations in genes associated with the three main regulatory pathways of autophagy or the autophagic process itself, e.g. those encoding: mTOR: DDIT-4 (DNA damage-inducible transcript 4), BRSK2 (BR Serine/Threonine Kinase 2), CDH23 (cadherin 23), PREX2 (Phosphatidylinositol-3,4,5-Trisphosphate Dependent Rac Exchange Factor 2), TOP3B (DNA Topoisomerase III Beta) , or RAC1: KALRN (Kalirin), EPHA2 (Ephrin Receptor EphA2), PLXND1 (plexin D1), ITGA3 (Integrin Subunit Alpha 3), DOCK6 (Dedicator Of Cytokinesis 6), GRK4 (G Protein-Coupled Receptor Kinase 4), EPHB 6 (Ephrin Receptor EphB6), or autophagy: ANXA5 (annexin A5), SEC31B (SEC31 homolog B, COPII coat complex component), DMXL2 (DMX - Drosophila melanogaster like 2), ATG9B (Autophagy related 9B), UNC93B (Unc-93 C. Elegans homolog B1) [11,12,57]. Other abbreviations: TLRs: Toll Like Receptors; NOD2: Nucleotide-binding and oligomerization-domain containing 2; CXCR4: C-X-C Motif Chemokine Receptor 4, receptor of CXCL12 or SDF-1: stromal cell-derived factor 1; TCR: T-cell receptor; IL-2/R: interleukin 2 and its receptor; PI3K: Phosphatidylinositol 3 kinase; RIPK2: Receptor-Interacting Serine/threonine Kinase; G α i : G alpha-i protein; ZAP70 (Zeta Chain Of T Cell Receptor Associated Protein Kinase 70); LCK: lymphocyte specific protein kinase; ERK (MAPK1): Extracellular signal-regulated kinase 1; AKT : serine/threonine kinase 1; BTNL2: butyrophilin like 2 protein; TAK1: Transforming growth factor beta-activated kinase 1 adaptor; TBK1: TANK binding kinase; RAS: rat sarcoma viral oncogene homolog; MKP1: MAP Kinase phosphatase 1; RHEB: Ras Homolog Enriched in Brain; TSC2: tuberlin or Tuberous Sclerosis 2 gene; VAV: Vav Guanine Nucleotide Exchange Factor 1; AKAP: A kinase anchoring protein; PKA: protein kinase A; JNK: JNK/MAPK8: Mitogen-activated protein kinase 8; mTOR: mammalian target of rapamycin; RAC1: Ras Related Botulinum Toxin Substrate 1; STAT1: Signal Transducer and Activator of Transcription 1; ULK1: UNC51 like kinase 1; LC3II: microtubule-associated protein light chain 3; NF κ B: Nuclear Factor Kappa B; IL-17A: interleukin 17A; IFN γ : gamma interferon; interleukins IL-6 and IL-8; CXCL1: chemokine (C-X-C motif) ligand 1; MIP2: macrophage inflammatory protein 2 or CXCL2; CCL2: chemokine ligand 2; RANTES: Regulated upon Activation, Normal T cell Expressed, and Secreted

Figure 3. Major mechanisms involved in the formation of granulomas . Under the influence of microbial or non-microbial triggers, tissue inflammation leads to activation of CD4⁺ T cells undergoing a chemotactic attraction towards inflamed tissues, inducing lymphopenia and participating in anergy induction. These inflammatory triggers induce tissue resident macrophage activation. IFN γ and IL-17A produced by activated Th1 and Th17 T lymphocytes drive macrophage M1 polarization. These cytokines act through specific receptors which induce different pathways and mainly JAK/STAT, mTOR, RAC1 related networks. Activated macrophages produce another set of pro-inflammatory cytokines, TNF α ,

IL-1 β , IL-6 and IL-13 that lead to aggregation of epithelial cells and further induction of multinucleated giant cells. Interactions with fibroblasts and surrounding T and B lymphocytes build the granuloma. The three (JAK/STAT, mTOR and RAC1) pathways regulate autophagy and it may be hypothesized that pathogenic decrease of autophagy decrease the clearance of external triggers (microbial or non-microbial) and enhance the inflammatory process both at the macrophage and T-cell levels. TH17: T helper cells; Treg: Foxp3⁺ T regulatory cells; IFN γ : gamma interferon; IFN γ -R: gamma interferon receptor; IL17A: interleukin 17A; JAK: Janus Kinase; STAT1: Signal Transducer and Activator of Transcription 1; TSC2: Tuberin or Tuberous Sclerosis 2; mTOR: Mechanistic Target of Rapamycin; Rac1: Ras Related Botulinum Toxin Substrate 1; TNF α : Tumor Necrosis Factor alpha; interleukins: IL-1 β : 1 β ; IL-6: IL-13; M-CSF: monocyte colony-stimulating factor; IL-2, IL-23: interleukin 23; TGF β : Transforming Growth Factor β ; ICAM: Intercellular Adhesion Molecule; DCSTAMP: Dendrocyte Expressed Seven Transmembrane Protein; MGC: multinucleate giant cells; B: B lymphocytes; F: fibroblasts; MBT: *Mycobacterium Tuberculosis*; SAA: Serum Amyloid Protein A; NNP: nanoparticles; GPCR: G protein coupled receptors; PD1/PDL1: Programmed death-1/programmed death ligand 1.

Abbreviations

AKAP: A kinase anchoring protein

AKT: serine/threonine kinase 1

AM: alveolar macrophage

AMBRA1: autophagy/beclin-1 regulator 1

AMPK: AMP-activated protein kinase

ANXA5: annexin A5

ATG9B: autophagy related 9B

ATG13: autophagy related 13

ATG14: autophagy related 14

ATG16: autophagy related 16

AZT: Azathioprine

BAL: bronchoalveolar lavage

BRSK2: BR Serine/Threonine Kinase 2

BTNL2: butyrophilin like 2 protein
cAMP: cyclic adenosine mono phosphate
CCL20: Chemokine CC motif ligand 20
CDH23: cadherin 23
CXCL9, 10, 11: chemokine CXC motif ligand 9, 10, 11
CD: Crohn Disease
CD4+: cluster differentiation 4 T cell
CD28: cluster differentiation
CTLA4: cytotoxic T-lymphocyte-associated protein 4
CXCL1: chemokine C-X-C motif ligand 1
CXCR4: C-X-C Motif Chemokine Receptor 4
DDIT4: DNA damage-inducible transcript4
DMXL2: DMX - Drosophila melanogaster like 2
DOCK6: Deducator of Cytokinesis 6
EC: epithelioid cell
EPHA2: Ephrin Receptor EphA2
EPHB6: Ephrin Receptor EphB6
ER: endoplasmic reticulum
ERK (MAPK1): Extracellular signal-regulated kinase 1
FOXP3: Forkhead box P3
G α i: G-alpha-i-protein
GAP: guanosine activating protein
GEF: guanosine exchanging factor
GMCSF, MCSF: granulocyte/macrophage colony stimulating factor and monocyte colony stimulating factor
GRK4: G protein-coupled receptor Kinase 4
HLADR: Major Histocompatibility Complex Class II
ICAM-1: Intercellular Adhesion Molecule 1

IF γ : gamma interferon

IL-1 β : Interleukin-1-beta

IL-4: Interleukin-4

IL-6: Interleukin-6

IL-13: Interleukin-13

IL-17A: Interleukin-17-A

IL-23: Interleukin-23

IL-2R: Interleukin 2 receptor

IRAK: Interleukin Receptor-associated kinase

ITGA3: Integrin Subunit Alpha 3

JAK: Janus Kinase

JNK/MAPK8: Mitogen-activated protein kinase 8

KALRN: Kalirin

LC3II: microtubule-associated protein light chain 3II

LCK: lymphocyte specific protein kinase

MAP: mitogen-activated-protein

MBT: mycobacterium tuberculosis

MGCs: multinucleate giant cells

MHC: Major Histocompatibility Complex

MIP2: macrophage inflammatory protein 2

MKP1: MAPK phosphatase

mTOR: Mechanistic Target of Rapamycin

mTORC1: mTOR related Complex 1

MWCNT: Multi wall carbon nanotubes

MYD88: Myeloid differentiation primary response protein

NADPH oxidase: Nicotinamide-Adenine Dinucleotide-phosphorylated

NF κ B: Nuclear Factor Kappa B

NNP: nanoparticles

NLRs: Leucine rich repeat receptors

NOD2: Nucleotide-binding and oligomerization-domain containing 2

PAMPs: Pathogen associated associated molecular patterns

PBMCs: peripheral blood mononuclear cell

PD1/PDL1: programmed death 1/programmed cell death ligand 1

PIK3CA: Phosphatidylinositol 3 kinase catalytic alpha

PPD: purified derivative protein

PKA: protein kinase A

PLXND1: Plexin D1

PREX1: phosphatidylinositol 3,4,5-triphosphate-dependent RAC exchanger 1 protein

PRRS: pathogen recognition receptors

QDOTs NNP: Quantum DOTs nanoparticles

Rac1: Ras Related Botulinum Toxin Substrate 1

RANTES: Regulated upon Activation, Normal T cell Expressed, and Secreted

RHEB: Ras Homolog Enriched in Brain

Rho-GTPase: Ras homologous GTPase

RIPK2 (RIP2): Receptor-Interacting Serine/threonine Kinase 2

ROR γ t: Retinoic-acid-receptor-related Orphan nuclear Receptor gamma

SAA: Serum Amyloid Protein A

SDF-1: stromal cell-derived factor 1

SEC31B : SEC31 homolog B, COPII coat complex component

STAT1: Signal Transducer and Activator of Transcription 1

STAT3: Signal Transducer and Activator of Transcription 3

STAT6: Signal Transducer and Activator of Transcription 6

TAK1: Transforming growth factor beta-activated kinase 1 adaptor

TBK1: TANK binding kinase; RAS: rat sarcoma viral oncogene homolog

T cells: T lymphocytes

TCR: T-cell antigen receptor

Th1/Th17: T helper cells

TGFβ: Transforming Growth Factor bêta

Tiam1: T-cell lymphoma invasion and metastasis-inducing protein-1

TLRs: Toll Like Receptors

TNFα: Tumor Necrosis Factor alpha

TOP3B: DNA Topoisomerase III Beta

TORC1: mTOR complex 1

Treg: Foxp3⁺ T regulatory cells

Tsc2: Tuberin or Tsc2 Gene

Tsc1: Hamartin or Tsc1 Gene

UBA: Ubiquitin-associated domain

ULK1: UNC51 like kinase 1

UNC93B: Unc-93 C. Elegans homolog B1

Vps34/15: Vacuolar Protein Sorting 34/15

WES: Whole Exome Sequencing

ZAP70: Zeta Chain of T Cell Receptor Associated Protein Kinase 70

GLOSSARY

M2 macrophage polarization: alternatively activated anti-inflammatory macrophages

Missing heritability : refers to the problem whereby genetic variants in genotype analysis cannot completely explain the heritability of complex traits; e.g. single genetic variations cannot account for much of the heritability of diseases, behaviors, and other phenotypes.

Non-caseating granulomas: granuloma without caseous necrosis

Xenophagy: type of selective macro-autophagy used for eliminating invading pathogens

BOX 1

AUTOPHAGY

Autophagy is an innate defense mechanism acting as a cell-autonomous system for the elimination of intracellular pathogens and foreign bodies (including bacteria, parasites, viruses and NNP [17–19, 61]. Autophagy promotes cellular senescence and cell surface antigen presentation, protects against genome instability and prevents necrosis, giving it a key role in preventing diseases such as cancer, neurodegenerative disorders, cardiomyopathy, diabetes, autoimmune diseases and infections. This “housekeeping” process is involved in both host defense and regulation of inflammation. The regulation of autophagy is under the strict control of a specialized protein network including ATG related proteins (ATG). The molecular machinery of autophagy includes multiple steps. The initiation step leads to the formation of the phagophore, in close vicinity with the endoplasmic reticulum (ER). This first step requires the ULK1/2-ATG13- FAK Family Kinase-Interacting Protein 200-KD (FIP200) complex, which is regulated by the autophagy suppressor TOR complex 1 (mTORC1). The second step of vesicle nucleation requires the Beclin1- Vacuolar Protein Sorting 34-15 (Vps34-Vps15)-ATG14 complex and promote membrane elongation. The third step, closure and maturation, associates with engulfment of cargo molecules by double-membrane vesicles, called autophagosomes, transported by the microtubule system. In a fourth step, late endosomes fuse with lysosomes, forming autolysosomes in which cargos are sequestered and degraded by the action of lysosomal hydrolases. Active at a basal level, autophagy is upregulated following a number of stimuli and stress conditions: amino acid deprivation, serum starvation and growth factor deprivation, hypoxia, exposure to various chemicals and toxins. Inducing signals converge at the level of mTOR protein complexes (mTORC1) that coordinate anabolic and catabolic processes. Under nutrient-rich conditions, mTORC1 limits the level of autophagy activity through inactivation of the ULK1/2 autophagy complex.

BOX 2

mTOR regulates autophagy

The mTOR kinase complex is a key component that regulates the balance between growth and autophagy in response to cellular physiological conditions and environmental stress [16,24,25]. It is negatively regulated by an upstream complex, TSC1–TSC2 (Tuberous Sclerosis Complex or HAMARTIN and TUBERIN, respectively) through its GTPase-activating protein activity towards a small G-protein, Rheb (Ras homologue enriched in brain) [16,26]. Inhibition of autophagy by mTOR works through an

initial step of this process involving AMBRA1 (autophagy/beclin-1 regulator 1) and various ULK (unc-51 like autophagy activating kinase).

The PI3K/AKT/mTOR pathway is a powerful inhibitor of autophagy by inactivation of ULK1 and autophagosome formation [114]. Following amino-acid starvation or mTOR inhibition, the activated ULK1 phosphorylates Beclin-1 and stimulates autophagosome formation. There are pathogenic variants of genes encoding regulators of mTOR [22]. For example, DDIT4 and DDIT4L (DNA damage-inducible transcript 4/L) are negative regulators of mTORC1 and modulate the phosphorylation of AKT [115]. Similarly, BRSK2 (Serine/Threonine Kinase 2) inhibits mTORC1 activity in a TSC2-mediated manner [116].

The NLRs (NOD1/2) related pathway activates NF- κ B through RIPK2 (Receptor-interacting serine/threonine-protein kinase 2), TAK1 (TGF- β -activated kinase 1) and TBK1 (Tank or Tumor necrosis factor receptor-associated family member-associated NF-kappa-B activator Binding Kinase 1) [117] (Figure 2B).

BOX 3

Whole Exome Sequencing of multifactorial diseases

Next generation sequencing (NGS) describe a DNA sequencing technology which allows whole exome (WES) or genome (WGS) analysis of genomic sequences. This new technology has revolutionized genomic research and the diagnosis of inherited diseases. In addition to an accurate and rapid analysis of genetic variants responsible for hereditary diseases, the WES method makes it possible to characterize genes sharing rare and/or frequent pathogenic variants associated to complex multifactorial diseases such as neurological and immunity-related syndromes. Many single nucleotide polymorphisms (SNPs) and copy number variations (CNVs) are associated with disease. In multifactorial diseases such as sarcoidosis, genetic, infectious and environmental factors are closely linked; hence, it is more necessary to identify a functional deficit in a molecular pathway(s) that may explain the sensitivity of patients to various environmental aggressions, rather than characterization of a single gene. To overcome the issue of missing heritability in multifactorial diseases, different strategies to unravel the genetic complexity can be applied. These include i) a select focus on sequencing high susceptibility individuals with young age-at-onset, or with positive family history [8],

ii) analyzing a cohort of stringent, well-phenotyped families and the availability of unaffected control subjects, either in a cohort of the general population, or preferably from healthy relatives in a predisposed family with accessible sibships, iii) pooling of rare variants for analysis, and iv) elucidating the gene regulatory network of identified disease-genes by functional pathway gene analyses [7, 9]. Pathogenic effects of gene variants can be evaluated by using a series of online available software as SIFT, POLYPHENv2, Mutation TASTER, PROVEAN or more complete commercial systems as ALAMUT VISUAL. The list of candidate genes thus produced can then be analyzed by enrichment methods using PPI (Protein-Protein Interaction) networks databases and functionally-related gene sets, such as gene ontology (GO) categories, suggesting that member genes are involved in a common process.

BOX 4

PHARMACOLOGICAL TARGETS IN SARCOIDOSIS

Autophagy and its two regulatory pathways Rac1 and mTOR are targeted by some of the therapeutic regimens proposed in steroid-resistant forms of sarcoidosis in humans. Proliferation of immune cells requires anabolic reactions and the synthesis of nucleic acids and proteins, which is mediated by activation of mTOR signaling [118]. Anti-metabolites such as Azathioprine that shows efficacy in some sarcoidosis patients interfere with these anabolic processes by up regulating autophagy via mTORC1 and the Unfolded Protein Response (UPR) (127). Glucocorticoids such as dexamethasone consistently induce autophagy in various cells and disease settings [119, 120]. Methotrexate inhibits DihydroFolate Reductase (DHFR), which is needed for amino acid and nucleic acid synthesis under nutrient-rich conditions and induces autophagy [121]. Leflunomide inhibits S6K1 (Ribosomal Protein S6 kinase 1), an mTORC1 substrate [122]. Azathioprine inhibits purine synthesis and can also block Rac1 signaling [28]. Chloroquine, used in cutaneous forms of sarcoidosis, targets lysosomes and interferes with autophagy, potentially blocking human blood CD4⁺ purified T cell [123]. Antibodies against TNF- α are used in severe forms of sarcoidosis and a close functional link between TNF receptors and the PI3/KT/mTOR pathway has been previously described [124, 125]. Nevertheless, the therapeutic effect of anti-TNF drugs remains obscure in some patients, and they may induce sarcoid-like lesions [126]. These pharmacological observations suggest that an induction of autophagy occurs in many therapeutic processes with the possibility of differential actions depending of the cellular context, T cells or macrophages.

REFERENCES

- 1 Valeyre, D. *et al.* (2014) Sarcoidosis. *Lancet* 383, 1155–1167
- 2 Broos, C.E. *et al.* (2013) Granuloma formation in pulmonary sarcoidosis. *Front. Immunol.* 4, article 437, 1-9
- 3 Petersen, H.J. and Smith, A.M. (2013) The role of the innate immune system in granulomatous disorders. *Front. Immunol.* 24; 4:120. doi: 10.3389/fimmu.2013.00120. eCollection 2013
- 4 Bento, C.F. *et al.* (2015) Autophagy in the fight against tuberculosis. *DNA Cell Biol* 34, 228–242
- 5 Esteves, T. *et al.* (2016) Is there any association between Sarcoidosis and infectious agents: a systematic review and meta-analysis. *BMC Pulm Med* 16: 165, doi 10.1186/s12890-016-0332-z
- 6 Vazeille, E. *et al.* (2015) Monocyte-derived macrophages from Crohn's disease patients are impaired in the ability to control intracellular adherent-invasive Escherichia coli and exhibit disordered cytokine secretion profile. *J. Crohns Colitis* 9, 410–420
- 7 Murrow, L. and Debnath, J. (2013) Autophagy as a stress-response and quality-control mechanism: implications for cell injury and human disease. *Annu. Rev. Pathol.* 8, 105–137
- 8 Cheng, Y. *et al.* (2013) Therapeutic targeting of autophagy in disease: biology and pharmacology. *Pharmacol. Rev.* 65, 1162–1197
- 9 Chen, Y. and Yu, L. (2017) Recent progress in autophagic lysosome reformation. *Traffic* 18, 358–361
- 10 Kabat, A.M. *et al.* (2016) The mucosal immune system and its regulation by autophagy. *Front. Immunol.* Jun 22; 7:240. doi: 10.3389/fimmu.2016.00240. eCollection 2016
- 11 Múzes, G. *et al.* (2013) Interplay of autophagy and innate immunity in Crohn's disease: a key immunobiologic feature. *World J. Gastroenterol.* 19, 4447–4454
- 12 Hampe, J. *et al.* (2007) A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. *Nat. Genet.* 39, 207–211
- 13 Ma, Y. *et al.* (2017) A Negative Feedback Loop Between Autophagy and Immune Responses in Mycobacterium leprae Infection. *DNA Cell Biol* 36, 1–9
- 14 Calender, A. *et al.* (2018) Whole exome sequencing in three families segregating a pediatric case of sarcoidosis. *BMC Med. Genomics* 11(1):23. doi: 10.1186/s12920-018-0338-x.
- 15 Besnard, V. *et al.* (2018) G908R NOD2 variant in a family with sarcoidosis. *Respir. Res.* 19(1):44. doi: 10.1186/s12931-018-0748-5
- 16 Linke, M. *et al.* (2017) Chronic signaling via the metabolic checkpoint kinase mTORC1 induces macrophage granuloma formation and marks sarcoidosis progression. *Nat. Immunol.* 18, 293–302

- 17 Damsky, W. *et al.* (2018) Tofacitinib treatment and molecular analysis of cutaneous sarcoidosis. *N. Engl. J. Med.* 379, 2540–2546
- 18 Manzia, T.M. *et al.* (2011) Successful treatment of systemic de novo sarcoidosis with cyclosporine discontinuation and provision of rapamune after liver transplantation. *Transpl Int* 24(8):e69-70. doi: 10.1111/j.1432-2277.2011.01256.x
- 19 Saxton, R.A. and Sabatini, D.M. (2017) mTOR Signaling in Growth, Metabolism, and Disease. *Cell* 168, 960–976
- 20 Rossides, M. *et al.* (2018) Familial aggregation and heritability of sarcoidosis: a Swedish nested case-control study. *Eur. Respir. J.* 52, pii: 1800385. doi: 10.1183/13993003.00385-2018
- 21 Fischer, A. and Rybicki, B.A. (2015) Granuloma genes in sarcoidosis: what is new? *Curr Opin Pulm Med* 21, 510–516
- 22 Calender, A. *et al.* (2019) Exome sequencing and pathogenicity-network analysis of 5 French families implicate mTOR signalling and autophagy in familial sarcoidosis. *Eur. Respir. J.* DOI: 10.1183/13993003.00430-2019
- 23 Kishore, A. *et al.* (2018) Whole-exome sequencing identifies rare genetic variations in German families with pulmonary sarcoidosis. *Hum. Genet.* 137, 705–716
- 24 Nguyen, L.K. *et al.* (2018) Rac1 and RhoA: Networks, loops and bistability. *Small GTPases* 9, 316–321
- 25 Shin, J.-Y. *et al.* (2016) Thiopurine Prodrugs Mediate Immunosuppressive Effects by Interfering with Rac1 Protein Function. *J. Biol. Chem.* 291, 13699–13714
- 26 Boissier, P. *et al.* (2013) EphA2 signaling following endocytosis: role of Tiam1. *Traffic* 14, 1255–1271
- 27 Koo, T.H. *et al.* (2007) Arf6 recruits the Rac GEF Kalirin to the plasma membrane facilitating Rac activation. *BMC Cell Biol.* 8, 29 doi: 10.1186/1471-2121-8-29
- 28 Xing, Y. *et al.* (2016) TLR and NLRP3 inflammasome-dependent innate immune responses to tumor-derived autophagosomes (DRibbles). *Cell Death Dis.* 7,e2322. Aug 4. doi: 10.1038/cddis.2016.206
- 29 Brown, J. *et al.* (2011) TLR-signaling networks: an integration of adaptor molecules, kinases, and cross-talk. *J. Dent. Res.* 90, 417–427
- 30 Kovach, M.A. and Standiford, T.J. (2011) Toll like receptors in diseases of the lung. *Int. Immunopharmacol.* 11, 1399–1406
- 31 Negroni, A. *et al.* (2018) NOD2 and inflammation: current insights. *J Inflamm Res* 11, 49–60
- 32 Travassos, L.H. *et al.* (2010) Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. *Nat. Immunol.* 11, 55–62

- 36 Carroll, B. *et al.* (2013). The TBC/ RabGAP Armus coordinates Rac1 and Rab7 functions during autophagy. *Developmental cell*, 25(1), 15–28.
- 33 Valentonyte, R. *et al.* (2005) Sarcoidosis is associated with a truncating splice site mutation in BTNL2. *Nat. Genet.* 37, 357–364
- 34 Pacheco, Y. *et al.* (2016) Familial vs. sporadic sarcoidosis: BTNL2 polymorphisms, clinical presentations, and outcomes in a French cohort. *Orphanet J Rare Dis* 11: 165. Dec 3. doi: 10.1186/s13023-016-0546-4
- 35 Swanson, R.M. *et al.* (2013) Butyrophilin-like 2 modulates B7 costimulation to induce Foxp3 expression and regulatory T cell development in mature T cells. *J. Immunol.* 190, 2027–2035
- 36 Malinowska, M. *et al.* (2017) Butyrophilins: an important new element of resistance. *Cent. Eur. J. Immunol.* 42, 399–403
- 37 Patterson, N.L. and Mintern, J.D. (2012) Intersection of autophagy with pathways of antigen presentation. *Protein Cell* 3, 911–920
- 38 Negi, M., *et al.* (2012). Localization of propionibacterium acnes in granulomas supports a possible etiologic link between sarcoidosis and the bacterium. *Modern Pathology : an official journal of the United States and Canadian Academy of Pathology, Inc*, 25(9), 1284–1297
- 39 Iio, K. *et al.* (2010) Experimental pulmonary granuloma mimicking sarcoidosis induced by Propionibacterium acnes in mice. *Acta Med. Okayama* 64, 75–83
- 40 Nakamura T, *et al.* (2016). Autophagy Induced by Intracellular Infection of Propionibacterium acnes. *PLoS One*.11(5), e0156298. doi: 10.1371/journal.pone.0156298
- 41 Zimmermann, A. *et al.* (2017) Atopobium and Fusobacterium as novel candidates for sarcoidosis-associated microbiota. *Eur. Respir. J.* 50, doi.org/10.1183/13993003.00746-2016.
- 42 Yu, T. *et al.* (2017) Fusobacterium nucleatum Promotes Chemoresistance to Colorectal Cancer by Modulating Autophagy. *Cell* 170, 548–563
- 43 Shui, W. *et al.* (2011) Organelle membrane proteomics reveals differential influence of mycobacterial lipoglycans on macrophage phagosome maturation and autophagosome accumulation. *J. Proteome Res.* 10, 339–348
- 44 Sakowski, E.T. *et al.* (2015) Ubiquitin 1 Promotes IFN- γ -Induced Xenophagy of Mycobacterium tuberculosis. *PLoS Pathog.* 11,e1005076. doi: 10.1371/journal.ppat.1005076. eCollection 2015
- 45 Chai, Q. *et al.* (2019) A Mycobacterium tuberculosis surface protein recruits ubiquitin to trigger host xenophagy. *Nat. Commun.* 10, 1973. doi: 10.1038/s41467-019-09955-8.
- 46 Huho, A. *et al.* (2017) The role of serum amyloid A staining of granulomatous tissues for the diagnosis of sarcoidosis. *Respir. Med.* 126, 1–8
- 47 Chen, E.S. *et al.* (2010) Serum amyloid A regulates granulomatous inflammation in sarcoidosis through Toll-like receptor-2. *Am. J. Respir. Crit. Care Med.* 181, 360–373

- 48 Zhu, S. *et al.* (2016). High-Density Lipoprotein (HDL) Counter-Regulates Serum Amyloid A (SAA)-Induced sPLA2-IIe and sPLA2-V Expression in Macrophages. *PloS one*, 11, :e0167468. doi: 10.1371/journal.pone.0167468. eCollection 2016
- 49 Pacheco, Y. *et al.* (2018) Granulomatous lung inflammation is nanoparticle type-dependent. *Exp Lung Res* 44, 25–39
- 50 Li, Y. and Ju, D. (2018) The Role of Autophagy in Nanoparticles-Induced Toxicity and Its Related Cellular and Molecular Mechanisms. *Adv. Exp. Med. Biol.* 1048, 71–84
- 51 Izbicki, G. *et al.* (2007) World Trade Center “sarcoid-like” granulomatous pulmonary disease in New York City Fire Department rescue workers. *Chest* 131, 1414–1423
- 52 Zhang, J. *et al.* (2018) Lysosomal deposition of copper oxide nanoparticles triggers HUVEC cell death. *Biomaterials* 161, 228-239. doi: 10.1016/j.biomaterials.2018.01.048.
- 53 Xia, L. *et al.* (2016) Endocytosed nanoparticles hold endosomes and stimulate binucleated cells formation. *Part Fibre Toxicol* 13, 63, doi: 10.1186/s12989-016-0173-1
- 54 Tattoli, I. *et al.* (2007) The nodosome: Nod1 and Nod2 control bacterial infections and inflammation. *Semin. Immunopathol.* 29, 289-301
- 55 Deretic, V. (2012) Autophagy as an innate immunity paradigm: expanding the scope and repertoire of pattern recognition receptors. *Curr. Opin. Immunol.* 24, 21–31
- 56 Delgado, M.A. *et al.* (2008) Toll-like receptors control autophagy. *EMBO J.* 27, 1110–1121
- 57 Wikén, M. *et al.* (2009) Higher monocyte expression of TLR2 and TLR4, and enhanced pro-inflammatory synergy of TLR2 with NOD2 stimulation in sarcoidosis. *J. Clin. Immunol.* 29, 78–89
- 58 Gabrilovich, M.I. *et al.* (2013) Disordered Toll-like receptor 2 responses in the pathogenesis of pulmonary sarcoidosis. *Clin. Exp. Immunol.* 173, 512–522
- 59 Veltkamp, M. *et al.* (2007) Linkage between Toll-like receptor (TLR) 2 promoter and intron polymorphisms: functional effects and relevance to sarcoidosis. *Clin. Exp. Immunol.* 149, 453–462
- 60 Crouser, E.D. *et al.* (2017) A novel in Vitro human granuloma model of sarcoidosis and latent tuberculosis infection. *Am. J. Respir. Cell Mol. Biol.* 57, 487-498
- 61 Locke, L.W. *et al.* (2019) IL-13-regulated Macrophage Polarization during Granuloma Formation in an In Vitro Human Sarcoidosis Model. *Am. J. Respir. Cell Mol. Biol.* 60, 84–95
- 62 Shamaei, M. *et al.* (2018) Evidence for M2 macrophages in granulomas from pulmonary sarcoidosis: A new aspect of macrophage heterogeneity. *Hum. Immunol.* 79, 63–69
- 63 Miyamoto, H. *et al.* (2012) An essential role for STAT6-STAT1 protein signaling in promoting macrophage cell-cell fusion. *J. Biol. Chem.* 287, 32479–32484

- 64 Wei, J.J. *et al.* (2019) Resolution of cutaneous sarcoidosis after Janus kinase inhibitor therapy for concomitant polycythemia vera. *JAAD Case Reports* 5, 360–361
- 65 Dickinson, J.D. *et al.* (2016) IL13 activates autophagy to regulate secretion in airway epithelial cells. *Autophagy* 12, 397–409
- 66 Sasaki, M. *et al.* (2001) Role of ICAM-1 in the aggregation and adhesion of human alveolar macrophages in response to TNF-alpha and INF-gamma. *Mediators Inflamm.* 10, 309–313
- 67 Tristão, F.S.M. *et al.* (2017) Th17-Inducing Cytokines IL-6 and IL-23 Are Crucial for Granuloma Formation during Experimental Paracoccidioidomycosis. *Front. Immunol.* 8, 949, doi: 10.3389/fimmu.2017.00949. eCollection 2017
- 68 Nishikawa, K. *et al.* (2014) Interleukin-17 induces an atypical M2-like macrophage subpopulation that regulates intestinal inflammation. *PLoS One* 9, e108494. doi: 10.1371/journal.pone.0108494. eCollection 2014
- 69 Facco, M. *et al.* (2011) Sarcoidosis is a Th1/Th17 multisystem disorder. *Thorax* 66, 144–150
- 70 Pacheco, Y. *et al.* (1982) Flow cytometry analysis of T lymphocytes in sarcoidosis. *Am. J. Med.* 73, 82–88
- 71 Grunewald, J. *et al.* (2002) Lung restricted T cell receptor AV2S3+ CD4+ T cell expansions in sarcoidosis patients with a shared HLA-DRbeta chain conformation. *Thorax* 57, 348–352
- 72 Hoshino, T. *et al.* (1995) Spontaneous production of various cytokines except IL-4 from CD4+ T cells in the affected organs of sarcoidosis patients. *Clin. Exp. Immunol.* 102, 399–405
- 73 Kaiser, Y. *et al.* (2019) Moving target: shifting the focus to pulmonary sarcoidosis as an autoimmune spectrum disorder. *Eur. Respir. J.* 2019 Jul 11;54(1). pii: 1802153. doi: 10.1183/13993003.021532018
- 74 Wahlström, J. *et al.* (2007). Identification of HLA-DR-bound peptides presented by human bronchoalveolar lavage cells in sarcoidosis. *The Journal of clinical investigation*, 117(11), 3576–3582
- 75 Kinloch, A.J. *et al.* (2018) In Situ Humoral Immunity to Vimentin in HLA-DRB1*03+ Patients With Pulmonary Sarcoidosis. *Front. Immunol.* Jul 9;9:1516. doi: 10.3389/fimmu.2018.01516
- 76 Kurebayashi, Y. *et al.* (2013). Recent advances in understanding the molecular mechanisms of the development and function of Th17 cells. *Genes to cells: devoted to molecular & cellular mechanisms*, 18(4), 247–265
- 77 Crouser, E.D. *et al.* (2018) Role of imbalance between Th17 and regulatory T-cells in sarcoidosis. *Curr. Opin. Pulm. Med.* 24: 521-526
- 78 Tateosian, N.L. *et al.* (2017) IL17A augments autophagy in Mycobacterium tuberculosis-infected monocytes from patients with active tuberculosis in association with the severity of the disease. *Autophagy* 13: 1191-1204

- 79 Astri, B. *et al.* (2015) Involvement of the IL-23/IL-17 axis and the Th17/Treg balance in the pathogenesis and control of autoimmune arthritis. *Cytokine* 74: 54-61
- 80 Judson MA. *et al.* (2012) Molecular profiling and gene expression analysis in cutaneous sarcoidosis: the role of interleukin-12, interleukin-23, and the T-helper 17 pathway. *J Am Acad Dermatol.*66(6):901-910
- 81 Ten Berge B. *et al.* (2012): Increased IL-17A expression in granulomas and in circulating memory T cells in sarcoidosis. *Rheumatology (Oxford)*. 51: 37-46
- 82 Toussirot E. *et al.* (2019) Safety of the use of anti-IL17A treatment in a patient with certolizumab-induced sarcoidosis. *Clin Exp Rheumatol.* 37(2):344-345
- 83 Liu, H. *et al.* (2013). Interleukin 17A inhibits autophagy through activation of PIK3CA to interrupt the GSK3B-mediated degradation of BCL2 in lung epithelial cells. *Autophagy*, 9(5), 730–742
- 84 Subramaniam SV. *et al.* (1999) Evidence for the involvement of JAK/STAT pathway in the signaling mechanism of interleukin-17. *Biochem Biophys Res Commun.*262(1):14-19.
- 85 Hubbard, V.M. *et al.* (2010) Macro-autophagy regulates energy metabolism during effector T cell activation. *J. Immunol.* 185, 7349–7357
- 86 Lee, N.-S. *et al.* (2011) Low levels of NF-κB/p65 mark anergic CD4+ T cells and correlate with disease severity in sarcoidosis. *Clin. Vaccine Immunol.* 18, 223–234
- 87 Chi, H. (2012) Regulation and function of mTOR signalling in T cell fate decisions. *Nat. Rev. Immunol.* 12, 325–338
- 88 Watanabe, N. *et al.* (2016) Induced Treg Cells Augment the Th17-Mediated Intestinal Inflammatory Response in a CTLA4-Dependent Manner. *PLoS One* 11, e0150244. doi: 10.1371/journal.pone.0150244. eCollection 2016
- 89 Handa T, *et al* (2003) Cytotoxic T-lymphocyte antigen-4 (CTLA-4) exon 1 polymorphism affects lymphocyte profiles in bronchoalveolar lavage of patients with sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis.* 20, 190-196
- 90 Tissot C. *et al.* (2012) Sarcoidosis complicating anti-cytotoxic T-lymphocyte associated antigen-4 monoclonal antibody biotherapy. *Eur Respir J.* 41, 246-7. doi: 10.1183/09031936.00107912
- 91 Paolini, L. *et al.* (2018) Thoracic and cutaneous sarcoid-like reaction associated with anti-PD-1 therapy: longitudinal monitoring of PD-1 and PD-L1 expression after stopping treatment. *J. Immunother. Cancer* 52, doi: 10.1186/s40425-018-0372-4

- 92 Habib, S. et al (2018) PDL-1 Blockade Prevents T Cell Exhaustion, Inhibits Autophagy, and Promotes Clearance of *Leishmania donovani*. *Infect Immun.* 86, pii: e00019-18. doi: 10.1128/IAI.00019-18.
- 93 Drent, M. et al. (2001) NF-kappaB activation in sarcoidosis. *Sarcoidosis Vasc. Diffuse Lung Dis.* 18, 50–56
- 94 Rosenbaum, J. T. et al. (2009). Hypothesis: sarcoidosis is a STAT1-mediated disease. *Clinical Immunology (Orlando, Fla.)*, 132(2), 174–183
- 95 Nemoz, G. et al. (1993) Impaired G-proteins and cyclic nucleotide phosphodiesterase activity in T-lymphocytes from patients with sarcoidosis. *Eur. J. Clin. Invest.* 23, 18–27
- 96 Talreja, J. et al. (2016) Dual Inhibition of Rip2 and IRAK1/4 Regulates IL-1 β and IL-6 in Sarcoidosis Alveolar Macrophages and Peripheral Blood Mononuclear Cells. *J. Immunol.* 197, 1368–1378
- 97 Rastogi, R. et al. (2011) Dysregulation of p38 and MKP-1 in response to NOD1/TLR4 stimulation in sarcoid bronchoalveolar cells. *Am. J. Respir. Crit. Care Med.* 183, 500–510
- 98 Seif, F. et al. (2017) The role of JAK-STAT signaling pathway and its regulators in the fate of T helper cells. *Cell Commun. Signal.* 15: 23, doi: 10.1186/s12964-017-0177-y.
- 99 Zhou, T. et al. (2017) Identification of Jak-STAT signaling involvement in sarcoidosis severity via a novel microRNA-regulated peripheral blood mononuclear cell gene signature. *Sci. Rep.* 7:4237, doi: 10.1038/s41598-017-04109-6.
- 100 Goldberg, A.A. et al. (2017) Regulation of ULK1 expression and autophagy by STAT1. *J. Biol. Chem.* 292, 1899–1909
- 101 Dillenburg-Pilla, P. et al. (2015) SDF-1/CXCL12 induces directional cell migration and spontaneous metastasis via a CXCR4/Gai/mTORC1 axis. *FASEB. J.* 29, 1056-1068
- 102 Kurdi, A.T. et al. (2016) Tiam1/Rac1 complex controls Il17a transcription and autoimmunity. *Nat. Commun.* 7:13048, doi: 10.1038/ncomms13048.
- 103 Yokosuka T. et al. (2010) The immunological synapse, TCR microclusters and T cell activation. *Curr Top Microbiol Immunol.* 340: 81-107
- 104 Roberts SD, et al. (2005). CD4+CD28-T cells are expanded in sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis.* 22, 13-19

- 105 Kremer, K.N. *et al.* (2017) TCR-CXCR4 signaling stabilizes cytokine mRNA transcripts via a PREX1-Rac1 pathway: implications for CTCL. *Blood* 130, 982–994
- 106 Saci, A. *et al.* (2011) Rac1 regulates the activity of mTORC1 and mTORC2 and controls cellular size. *Mol. Cell* 42, 50–61
- 107 Carroll B, *et al* (2013) The TBC/RabGAP Armus coordinates Rac1 and Rab7 functions during autophagy. *Dev Cell*. 25, :15-28 doi: 10.1016/j.devcel.2013.03.005. Epub 2013 Apr 4.
- 108 Hooper KM. *et al.* (2019) The Inflammatory Bowel Disease Drug Azathioprine Induces *Autophagy via mTORC1 and the Unfolded Protein Response Sensor PERK*. *Inflamm Bowel Dis.*, 25, 1481-1496.
- 109 Molitoris, J.K. *et al.* (2011) Glucocorticoid elevation of dexamethasone-induced gene 2 (Dig2/RTP801/REDD1) protein mediates autophagy in lymphocytes. *J. Biol. Chem.* 286, 30181–30189
- 110 Xu, K. *et al.* (2015) Autophagy induction contributes to the resistance to methotrexate treatment in rheumatoid arthritis fibroblast-like synovial cells through high mobility group box chromosomal protein 1. *Arthritis Res. Ther.* 17:374, doi: 10.1186/s13075-015-0892-y.
- 111 Sun, J. *et al.* (2018) Inhibition of p70 S6 kinase activity by A77 1726 induces autophagy and enhances the degradation of superoxide dismutase 1 (SOD1) protein aggregates. *Cell Death Dis.* 9, 407 doi: 10.1038/s41419-018-0441-0.
- 112 Schmidt, R.L.J. *et al.* (2017) Chloroquine inhibits human CD4+ T-cell activation by AP-1 signaling modulation. *Sci. Rep.* 7, 42191 doi: 10.1038/srep42191.
113. Crommelin, H.A. *et al.* (2014) Anti-TNF therapeutics for the treatment of sarcoidosis. *Immunotherapy.* 6, 1127–1143
- 114 Vigne, C. *et al.* (2013) Sarcoidosis: An underestimated and potentially severe side effect of anti-TNF-alpha therapy. *Joint Bone Spine.* 80, 104–107

DISEASES

Tuberculosis
Crohn's disease
Leprosy
Sarcoidosis

TRIGGERS

Bacteria
Viruses
SAA
Nutrients
Nanoparticles

EXPERIMENTAL MODELS

Tsc2^{-/-} mouse model
IL-13 in vitro
Nanoparticles in vivo

GRANULOMA

AUTOPHAGY







