Commentary

Loss of C9orf72 function leads to autoimmunity

Lazaros I. Sakkas[1], Dimitrios P. Bogdanos[1], Eleni E. Kousvelari[2]

[1]Department of Rheumatology and clinical Immunology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa, Greece; [2]Dental School, National and Kapodistrian University of Athens, Athens, Greece

Correspondence to: Lazaros I. Sakkas, MD, DM, PhD (UK), FRCP (UK). Department of Rheumatology and Clinical Immunology, Faculty of Medicine, Larissa 41 110, Greece. Email: lsakkas@med.uth.gr.

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A widely held view for the etiology of autoimmune diseases is that environmental factors combined with a proper genetic background can cause the disease. Rare monogenic diseases with autoimmune manifestations combined with new technologies, such as whole exon genomic sequencing, has unearthed fascinating mechanisms of autoimmune diseases. One such an example is the coatomer subunit alpha (COPA) syndrome which is caused by functionally defective variants of the *COPA* gene. COPA variants impair the retrograde transport of proteins from Golgi to endoplasmic reticulum (ER), increase ER stress and defective autophagy and lead to Th17 induction and expansion (1). COPA syndrome is manifested with interstitial lung disease, lung hemorrhages, arthritis, many autoantibodies, including antinuclear antibodies (ANA), anti-neutrophil cytoplasmic antibodies (ANCA), and rheumatoid factor (RF), Th17 immune response, and, in lung biopsies, germinal center formation (1).

Recently, mutations of chromosome 9 open reading frame 72 (*C9ORF72*) gene, apparently involved membrane cytoplasmic trafficking, and particularly autophagy, have been shown to cause autoimmune manifestations (2). *C9ORF72* gene mutations have been associated with amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The most commonly found mutation is a hexanucleotide GGGGCC repeat expansion in an intron of the gene and it has been proposed that the hexanucleotide repeat expansion silences the mutant allele (3). The exact mechanism for the associations with ALS/FTD was not known, since the function of the *C9ORF72* gene product, C9orf72, was not elucidated. However, in a recent paper Burberry *et al.* are beginning to shed some light into the mouse ortholog C9orf72 function (2). They created a C9orf72 mutant (–/–) mice after intercrossing heterozygous animals having exons 2–6 of the gene replaced by *LacZ* and neomycin resistance cassette and designated these as KOMP mutants. C9orf72 mutant (–/–) mice were also generated through homologous recombination of clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 targeting exon 4 of C9orf72, without neomycin resistance cassette that might have interfered with nearby genes (neo-deleted strain). Both KOMP (–/–) and (+/–) mutants had reduced survival, whereas neo-deleted strains lived longer. Loss-of-function of C9orf72 ortholog led to features of autoimmunity long before clinical manifestations and animals developed anemia, thrombocytopenia, neutrophilia, splenomegaly with T cell and B cell infiltrations, enlarged lymph nodes, elevated levels of pro-inflammatory cytokines [IL-6, IL-17, IL-22, monocyte chemoatractant protein-1 (MCP-1), granulocyte-macrophage colony stimulating factor (GM-CSF)] (2). Using autoantigen microarrays, Burberry *et al.* detected serum IgM and IgG autoantibodies against 117 of 124 and 113 of 124 autoantigens, respectively, in neo-deleted (–/–) mutants (2). These autoantibodies included antibodies against anti-dsDNA, which are highly specific for systemic lupus erythematosus (SLE), a prototypic systemic autoimmune disease. In (+/–) animals

the researchers found IgM autoantibodies against 1 of 124 autoantigens (KLM-1) and IgG autoantibodies against 2 of 124 autoantigens (collagen II and Mi2). Anti-LKM-1 antibodies are highly specific for autoimmune hepatitis type 2. The immune genome (ImmunGen) database indicated that *C9ORF72* and its murine ortholog were expressed in blood cells (4), and, based on that, Burberry *et al.* did transplantation experiments to confirm that C9orf72 mutant indeed causes autoimmunity. Transplantation of mutant (–/–) bone marrow cells to wild type (WT) animals caused autoimmunity and premature mortality, whereas transplantation of WT mice bone marrow cells into mutant (–/–) animals improved phenotype (2). This work is in agreement with the work of Atanasio *et al.* (5) who found that C9orf72 null mice developed myeloid expansion, T cell activation and increased plasma cells. C9orf72 null mice had autoantibodies and glomerulopathy, abnormalities reminiscent of SLE. Spleen and lymph node signature in C9orf72 (–/–) mice indicated myeloid cell infiltration (5). Similarly, two different lines of mice lacking the C9orf72 ortholog, developed splenomegaly and lymphadenopathy with accumulation of engorged macrophage-like cells. C9orf72 expression was highest in myeloid cells (6).

Bioinformatics analysis revealed that C9orf72 has structural homology to a family of proteins called DENN, differentially expressed in normal and neoplastic cells, which function as GDP/GTP exchange factors (GEF) of Rab GTPases implicated in autophagy (7,8). Autophagy is a major intracellular system by which cytoplasmic materials are delivered to lysosomes for degradation, but also produces building blocks, mainly aminoacids, for cell homeostasis. For instance, starvation of aminoacids induces autophagy to restore intracellular aminoacid levels, and when this is achieved, mammalian target of rapamycin complex 1 (mTORC1) is activated and terminates autophagy (9). Autophagy is selective or non-selective and is classified as macroautophagy, microautophagy and chaperone-mediated autophagy. Impairment of autophagy leads to accumulation of p62, a selective substrate of autophagy, which forms aggregates with ubiquitin (9). C9orf72 is a Rab1 effector that promotes initiation of autophagy, and disruption of C9orf72 function in cell lines and primary neurons inhibits autophagy and increased ER stress (10). This latter has been an expected finding since autophagy alleviates ER stress (11). C9orf72 also interacts with other Rabs (12). A close paralog of Rab39B, Rab39A, negatively regulates bacterial lipopolysaccharide (LPS)-induced autophagy in macrophages (13). Rab8B, a close paralog of Rab8A, acts through the innate immunity regulator TRAF family member-associated NF-κB activator (TANK)-binding kinase 1 (TBK-1), thus linking autophagy with innate immune response (14). TBK1 is a kinase that functions in interferon (IFN) signaling and autophagy and is able to cause ALS and FTD (15). Neutralization of TBK1 function leads to a phenotype very similar to C9orf72 neutralization. For instance, Cre-mediated removal of Tbk1 in T cells causes T cell activation and T cell infiltration of spleen and lymph nodes (16). Also, mice lacking TBK1 activity develop immune cell infiltrates in many organs and tissues, including skin, lung, liver and kidneys, and increased susceptibility to LPS-induced mortality (17). Defects in autophagy, called microtubule-associated protein light chain 3 (LC-3)-associated phagocytosis (LAP), also cause a SLE-like autoimmune disease. Mice lacking any of the LAP pathway components develop glomerulopathy with glomerular immune complex deposition, autoantibodies and inflammatory cytokines. In addition, repeated injections of dying cells into LAP-deficient, but not LAP-efficient mice exacerbated SLE-like disease (18).

So, what connects autoimmunity with ALS/FTD? O'Rourke *et al.* showed that C9orf72 is required for normal function of myeloid cells and microglia, since loss of C9orf72 led to lysosomal accumulation and altered immune responses in macrophages and microglia (6). Also, C9orf72 depletion in HeLa cells and neurons caused accumulation of p62-positive inclusion, thus mimicking p62 pathology in ALS/FTD (10).

There is growing body of evidence that autophagy cross-talks with immune system at multiple levels, such as T cell development, HLA-class II antigen presentation, inflammasome activation, and is implicated in the development of autoimmune diseases (19). The paper by Burberry *et al.* showing that defects of C9orf72, apparently involved in cytoplasmic trafficking, lead to autoimmune diseases provides new insights into pathogenesis of autoimmune diseases and opens a new avenue for novel therapeutic strategies in these diseases.

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None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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