6 Autoinflammatory Diseases Due to Defects in Degradation or Transport of Intracellular Proteins

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Abstract

The number of human inborn errors of immunity has now gone beyond 430. The responsible gene variants themselves are apparently the cause for the disorders, but the underlying molecular or cellular mechanisms for the pathogenesis are often unclear. In order to clarify the pathogenesis, the mutant mice carrying the gene variants are apparently useful and important. Extensive analysis of those mice should contribute to the clarification of novel immunoregulatory mechanisms or development of novel therapeutic maneuvers critical not only for the rare monogenic diseases themselves but also for related common polygenic diseases. We have recently generated novel model mice in which complicated manifestations of human inborn errors of immunity affecting degradation or transport of intracellular proteins were recapitulated. Here, we review outline of these disorders, mainly based on the phenotype of the mutant mice we have generated.

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6.1 Introduction

The immune defense of higher animals depends on the cooperation of innate and adaptive immunity. Adaptive immunity is mediated by lymphocytes such as B and T cells. They recognize a variety of molecular structures derived from pathogens, the so-called antigens, by means of immunoglobulins and T cell receptors. Although adaptive immunity is an elaborate system in terms of antigen specificity, it takes time to be established, and in the early stages of infection, innate immunity plays a major role in the immune response. Innate immunity is mediated by macrophages, dendritic cells, and neutrophils. These cells phagocytose or sense pathogens and produce inflammatory cytokines, including type I interferons (IFNs) to elicit inflammatory responses and eliminate pathogens. Macrophages and dendritic cells also play an important role in the establishment of adaptive immunity by inducing the activation and differentiation of T cells as antigen-presenting cells.

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Next-generation sequencing technology greatly contributed to identification of genetic variants responsible for various kinds of hereditary diseases, including immune disorders. The term, inborn error of immunity (IEI) is proposed to cover monogenic diseases manifesting a variety of immune disorders including autoimmunity, autoinflammation, and/or allergy. The number of inborn errors of immunity (IEI) has reached more than 430 in 2020 [1]. They are mostly caused by monogenic variants that lead to loss of function (LOF) or gain of function (GOF) of the encoded proteins. Immune disorders can be roughly categorized into deficiency or hyperactivation of innate or adaptive immunity. Surprisingly, however, monogenic variants often lead to mixed manifestations of those dysfunctions. We have generated two novel mutant mice in which the mixed manifestations of human inborn errors of immunity were recapitulated. One carries a novel gene variant in the subunit of proteasome, which is involved in protein homeostasis by degrading unnecessary or useless proteins and manifests increase of innate immune cells with adaptive immunodeficiency. The other carries a novel gene variant in the subunit of COPI, which is critical for retrograde transport of intracellular proteins and exhibits dysregulated cytosolic DNA sensor signaling with adaptive immune hyperactivation.

6.2 Autoinflammatory Disease

The term, autoinflammatory, was first used by Kastner in 1999 [2]. Kastner group identified missense mutations of *TNFRSF1A,* encoding the 55 kDa tumor necrosis factor receptor (TNFR1), responsible for the autosomal dominant periodic fever syndrome, TNFR1-associated periodic syndromes (TRAPS) [2]. Autoinflammatory diseases are primarily monogenic hereditary diseases manifesting mainly dysregulated innate immunity, in contrast to autoimmune diseases, which represent dysregulated adaptive immunity, although most of them are polygenic [3].

Familial Mediterranean fever (FMF) and cryopyrin-associated periodic fever syndrome (CAPS) are known as representative autoinflammatory diseases. The pathogenesis of FMF and CAPS mainly depends on the overactivation of pyrin and NLRP3 inflammasomes, respectively, which are required for cleavage of an immature inactive to a mature active form of a proinflammatory cytokine, IL-1β [4]. Overproduction of IL-1β is caused by GOF variants of those inflammasome subunits. Furthermore, LOF variants of the molecules regulating the inflammasomes are also being clarified. One example would be Hyper IgD syndrome. It is an autoinflammatory disease caused by LOF variants of mevalonate kinase (MVK), involved in the cholesterol metabolism [5, 6]. In steady states, pyrin inflammasome activity is negatively regulated by a Rho GTPase, RhoA, which is localized in the plasma membrane. MVK catalyzes generation of geranylgeranyl pyrophosphate, which is required for geranylation and membrane localization of RhoA. Without MVK, RhoA is released from the membrane and cannot negatively regulate the pyrin inflammasome [7].

In 1984, Aicardi and Goutières described an inherited disease characterized by calcification of the basal ganglia and lymphocyte proliferation in the cerebrospinal fluid, with findings resembling viral infection via the placenta [8]. This disease is now classified as a group of autoinflammatory diseases called Aicardi-Goutières syndrome (AGS). Gene variants for AGS were found in several genes encoding the molecules involved in intracellular degradation or sensing of nucleic acids [9–14]. Those gene variants cause dysregulated activation of nucleic acid sensors or type I IFN signaling and lead to exaggerated production or signaling of type I interferons (IFNs). The resultant pathological conditions are collectively called as type I interferonopathies [15, 16]. In addition, a GOF variant of the genes encoding stimulator of IFN genes (STING), which is an adapter molecule essential for the cytosolic DNA sensor pathway, also results in the increase of type I IFN production, called as STING-associated vasculopathy with onset in infancy (SAVI) [17]. Type I interferonopathy is also observed in some polygenic autoimmune diseases such as systemic lupus erythematosus (SLE).

There remain a number of autoinflammatory diseases, pathogenesis of which is still unknown. In the following chapters, we discuss those diseases which also manifest adaptive immune dysfunctions.

6.3 Proteasome-Associated Autoinflammatory Syndrome

In order to keep protein homeostasis, proteins should be degraded promptly when they are deteriorated or fail to achieve the correct higher-order structure. The degradation of such unnecessary or useless proteins involves a protein complex called proteasome (Fig. 6.1) [18, 19]. Proteasome is composed mainly of seven α and seven β subunits, which form α and β rings, respectively. Two α and two β rings are assembled as a αββα form, which is called the 20S core complex. Then several regulatory subunits are added to form the 26S proteasome, which functions as a mature proteasome (Fig. 6.1a). Proteasome activity is mediated by three β subunits, β1, β2, and β5 and the proteasome containing these three subunits is called as the constitutive proteasome, which is expressed in most cells (Fig. 6.1b). Cytokine-stimulated cells or most of hematopoietic cells contain β1i, β2i, and β5i, instead of β1, β2, and β5, respectively, and this form of proteasome is called as the immunoproteasome. Cortical thymic epithelial cells, which are involved in positive selection of CD8 T cells, express their specific subunit, β5t, and carry the thymoproteasome, which contains β5t as well as β1i and β2i [20]. Proteasome is involved not only in protein degradation but also in generation of MHC class I-restricted antigen peptides, thereby playing critical roles in generation and responses of cytotoxic T cells.

In 1939, Nakajo described a systemic autoinflammatory disease with progressive wasting as "secondary hypertrophic osteoperiostitis with pernio" in a brother and a sister cases of a consanguineous family [21]. In 1950, Nishimura also reported three cases with similar phenotype of two consanguineous families [22, 23]. Kitano

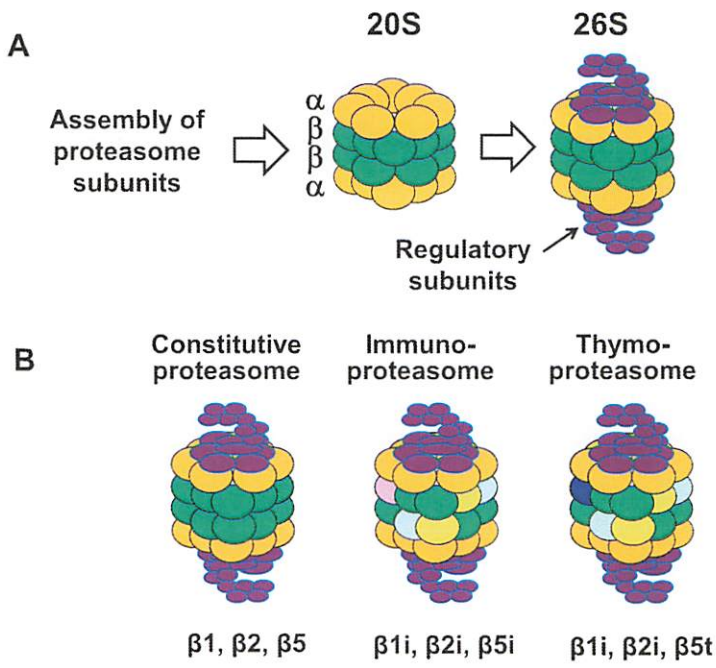

Fig. 6.1 Structure of proteasome. (**a**) Seven α and seven β subunits bind and form the α and β rings, respectively. Then two α and two β rings are assembled as a αββα form, which is called the 20S core complex. After the addition of several regulatory subunits, the 26S proteasome is generated as a mature proteasome. (**b**) The 26S proteasomes exist as constitutive proteasome, immunoproteasome and thymoproteasome. They carry their own β subunits with proteasome activity

further summarized 12 cases of eight families as a novel "syndrome with nodular erythema, elongated and thickened fingers, and emaciation" [24]. The disease was then named as Nakajo-Nishimura syndrome after the two discoverers and the responsible gene variant was identified as a homozygous missense variant (c.602G > T, p.G201V) in *PSMB8* encoding a proteasome subunit, β5i [25, 26]. Autoinflammatory diseases carrying other *PSMB8* variants have been reported and called by various names, including joint contractures, muscular atrophy, microcytic anemia, and panniculitis-induced lipodystrophy (JMP) syndrome or chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE) syndrome [27, 28]. Then gene variants of other proteasome subunits or proteasome-related chaperons were also found to cause similar autoinflammatory manifestations. Now diseases caused by these gene variants are collectively called as proteasome-associated autoinflammatory syndrome (PRAAS) [29]. In most cases, PRAAS patients carry monogenic homozygous or compound heterozygous variants of proteasome subunits, but some carry digenic heterozygous variants of the subunits [25–34] or monogenic heterozygous variants of proteasome-associated chaperon molecules [29, 35, 36]. Thus, PRAAS is an autoinflammatory disease caused by gene variants of proteasome subunits and their related molecules.

In PRAAS, proteasome assembly is impaired and the activity is decreased. Proteins degraded by the proteasome are ubiquitinated, so ubiquitin and ubiquitinated proteins accumulate in the tissues and cells of PRAAS patients. Several molecules were found to be responsible for elevated production of proinflammatory cytokines or type I interferons (IFNs) in PRAAS patients. Phosphorylated p38, a mitogen-activated protein kinase, was accumulated in PRAAS patient cells [25, 26]. Inhibitor for p38 decreased the production of proinflammatory cytokines such as IL-6 in myeloid cell lines from the patient-derived induced pluripotent stem cells, indicating the involvement of p38 [37]. THP-1, a human monocytic cell line, shows increased production of type I IFNs and proinflammatory cytokines in a protein kinase R (PKR)-dependent manner, when a proteasome subunit, β1i or β5i, is deleted or upon the treatment with proteasome inhibitors [38]. The PKR activation was found to be caused by intracellular accumulation of IL-24. Furthermore, the PKR inhibitor ameliorated increased expression of type I IFN-induced genes in PRAAS patient samples [38]. It is also notable that the homozygous *Psmb8 G201V* mutant mice show exaggerated manifestations of imiquimod-induced dermatitis, which was ameliorated by deficiency of a chemokine receptor, CXCR3, indicating the involvement of this chemokine system [39].

6.4 Phenotype of Proteasome Subunit-Deficient Mice

Various proteasome subunit-deficient mice have been generated and analyzed. In mice lacking all three β-subunits β1i, β2i, and β5i, which are responsible for proteolytic activity in the immunoproteasome, the pattern of antigen peptides presented by MHC class I is altered, which leads to decreased membrane surface expression of MHC class I, and CD8 T cell responses are impaired [40]. In mice lacking β5t in addition to β1i, β2i, and β5i (quadruple knock out mice), the number of CD8 T cells and their responses are also significantly impaired [41]. However, in both mutant mice, immune cells other than CD8 T cells are hardly impaired, and no inflammatory symptoms have been reported. Furthermore, the adaptive immunity of PRAAS patients is sometimes abnormally activated, which is indicated by detection of antinuclear antibodies, but CD8 T cells are rarely reduced. Thus, there is a discrepancy between the findings of PRAAS patients and the phenotype of proteasome subunit-deficient mice.

On the other hand, "emaciation" phenotype of the patients seems to be recapitulated in mice. Impaired adipocyte differentiation has been observed not only in β5i deficient mice but also in mice carrying the *Psmb8 G201V* mutation [39, 42]. It is, however, still unknown whether the PRAAS patients show similar defects in adipo-

cyte differentiation. Further studies are required to elucidate the molecular mechanisms how the defective immunoproteasome leads to impairment of adipocyte differentiation.

6.5 Proteasome-Associated Autoinflammatory Syndrome with Immunodeficiency

Two independent patients were found to show clinical manifestations similar to, but distinct from PRAAS [43]. Both patients presented periodic and refractory myositis and other inflammatory symptoms similar to PRAAS in the neonatal period, and basal ganglia calcification characteristic of PRAAS was observed on head CT. Meanwhile, the patients did not show any signs of lipoatrophy, which is usually seen in PRAAS, and they also had pulmonary hypertension and lymphocytopenia (adaptive immunodeficiency), which are rare in PRAAS. Exome analysis of these patients together with their parents revealed a de novo heterozygous missense variant (c.467G > A, p.G156D) in *PSMB9* encoding the proteasome subunit β1i as the same gene variant specific to the patients. This glycine is conserved across species not only among β1i but also among β1 and structural modeling analysis infers that it is located at the interface between two β-rings (Fig. 6.2a, b). It is noteworthy that the previous PRAAS gene variants are mostly found at or near the active site and that the active sites are conformationally disrupted. The variated glycine, however, is far from the active site and the active site structure is hardly affected by the substitution to aspartic acid. Furthermore, the proteasome activity defect is also different from that of PRAAS described so far. Both 20S and 26S proteasome activities are reduced and, as a result, ubiquitin accumulation was observed in typical PRAAS. Meanwhile, in the patient-derived cells, 26S proteasome activity was almost intact and ubiquitin accumulation was hardly detected, although proteasome formation such as β1i maturation was defective and 20S proteasome activity was decreased.

To further clarify the pathological significance of this gene variant, mutant mice carrying the variant were generated and analyzed [43]. The heterozygous mutant mice appeared healthy at a glance. In embryonic fibroblasts from the heterozygous mutant mice, β1i protein maturation was impaired and the 20S proteasome activity was reduced, while the 26S proteasome activity was retained and ubiquitin accumulation was not detected. This pattern of proteasome defect is similar to that of patient-derived cells. Immunological analysis further revealed decrease of T and B lymphocytes and dendritic cells and severe decrease in the serum of all isotypes of immunoglobulins. Meanwhile, neutrophils and monocytes were increased. Interestingly, the homozygous mutant mice also showed similar patterns of proteasome defects, such as decrease and retainment of the 20S and 26S proteasome activities, respectively, though they showed complete loss of T, B, and dendritic cells. Thus, the mice carrying the *Psmb9 G156D* manifested proteasome defects and adaptive immunodeficiency in similar to the patients, indicating that *PSMB9 G156D* is the causative gene variant.

It is noteworthy that *PSMB9 G156D* can cause the manifestations as a monogenic heterozygous variant in a dominant form of inheritance, while most of the gene variants responsible for PRAAS to date show a latent (recessive) form of inheritance. In the heterozygous *Psmb9 G156D* mice, MHC class I expression was not impaired and processing and MHC class I-restricted presentation of the ovalbumin protein was intact. This is in contrast to the findings that deficiency of β1i as well as all of four β subunits, β1i, β2i, β5i, and β5t in mice does not show a marked immunodeficiency but only a mild decrease in CD8 T cells due to defective expression of MHC class I. This suggests that *PSMB9 G156D* caused a functional modification rather than a mere loss of function. We can surmise that the variant lead to disruption of β ring association and 20S proteasome dysfunction, thereby impairing the mechanisms that maintain immune homeostasis while retaining protein degradation (Fig. 6.3). It is also notable that the immunodeficient phenotype is observed also in mice carrying a missense mutation of β2i,

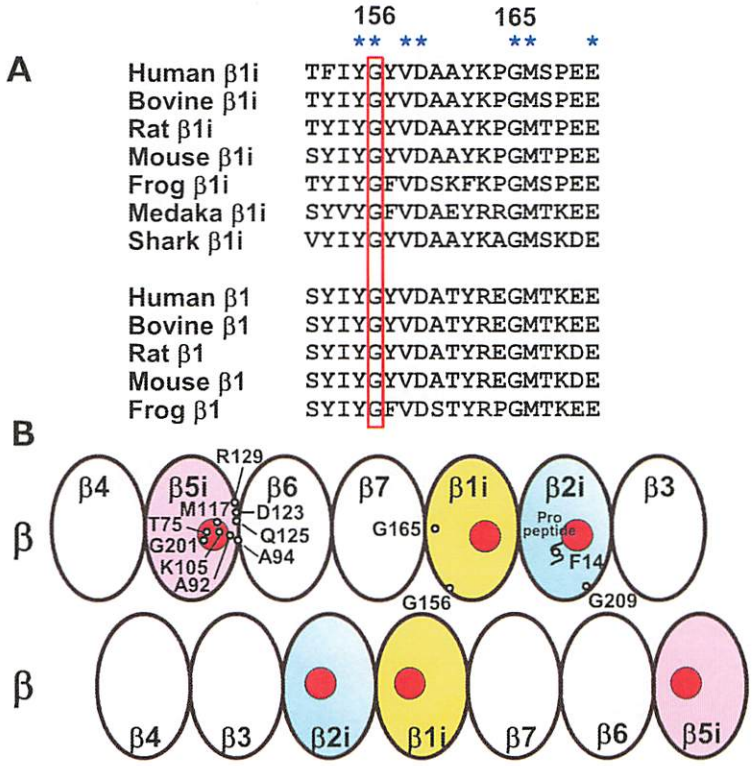

Fig. 6.2 Characterization of the proteasome β subunit variants. (**a**) Comparison of glycine at position 156 (boxed) of the proteasome β1i subunit and its surrounding amino acids among β1 and β1i from various species. Conserved amino acids are indicated by asterisks. (**b**) Locational distribution of published amino acid variants (https://infevers.umai-montpellier.fr/web/index.php) on the schematic model of β rings in the immunoproteasome. Catalytic active sites in β1i, β2i and β5i are marked with red circles. G156 of β1i is located in the interface of β rings, away from the active site. G209 of β2i is also located in the interface of β rings. Here G209 is numbered, based on the amino acid number from the first methionine, although it is described as G170 in the original paper [44]

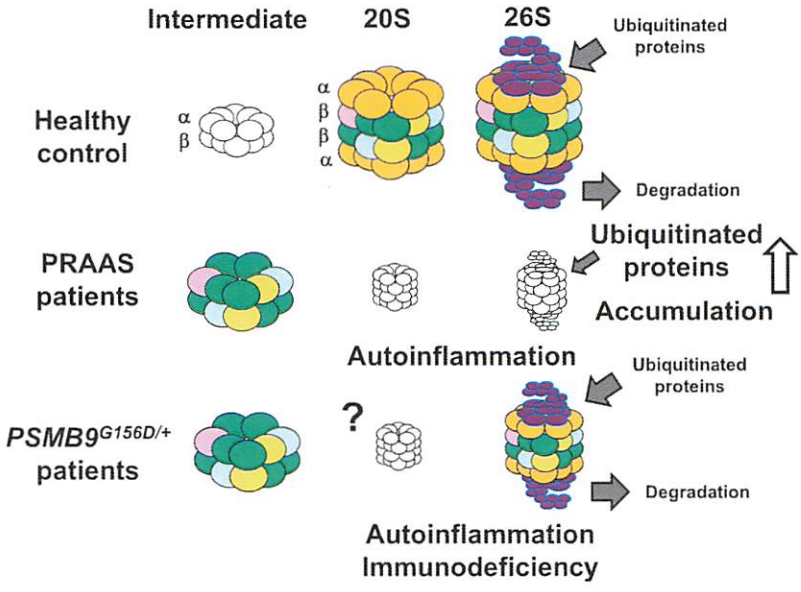

Fig. 6.3 Comparison of immunoproteasome assembly. In healthy control, 20S and 26S proteasomes are formed and few intermediates are detected. In PRAAS patients, formation of both 20S and 26S proteasomes is reduced and intermediates are increased. As a result, ubiquitinated proteins are accumulated, leading to autoinflammation. In patients with a heterozygous variant for *PSMB9 p.G156D*, the 20S proteasome activity is decreased and intermediates are increased, while the 26S proteasome activity is retained and accumulation of ubiquitinated proteins are hardly detected. However, the patients show not only autoinflammation but also adaptive immunodeficiency

Psmb10 G209W, which is introduced by N-ethyl-N-nitrosourea mutagenesis [44]. The heterozygous mutant mice showed defects of both CD4 and CD8 T cells and the homozygous mutant mice lacked B as well as T cells and manifested skin disorders with hyperkeratosis and infiltration of neutrophils. This amino acid, G209, is highly conserved among β2 and β2i across species and structural modeling analysis shows that it is located at the interface of the two β rings (Fig. 6.2b). Furthermore, this mutation also caused decrease of the 20S, while keeping the 26S proteasome formation. It can be assumed that these gene variants in the interface of the two β rings should impair the formation of the 20S complex and cause adaptive immunodeficiency. Further studies are necessary to clarify why the 26S proteasome formation is retained and why the defects of the 20S proteasome leads to adaptive immunodeficiency. Based on these findings, we have now proposed the concept of proteasome-associated autoinflammatory syndrome with immunodeficiency (PRAAS-ID) as a unique category of disease, similar to, but distinct from typical PRAAS.

A treatment for PRAAS has not yet been established, but it has been suggested that JAK inhibitors, which block cytokine signaling, are effective [45, 46]. It should also be noted that tofacitinib was effective in one PRAAS-ID patient [47]. In addition, cord blood transplantation was effective in this case, suggesting that hematopoietic cell abnormalities are important in the pathogenesis of PRAAS-ID.

6.6 COPA Syndrome

Intracellular proteins should be transported properly among various organelles such as the Golgi apparatus or the endoplasmic reticulum (ER) (Fig. 6.4). Secretary or membrane proteins are synthesized in the rough ER, transported through the Golgi apparatus to trans-Golgi network (TGN) and then secreted or reach the cell surface. Meanwhile, some proteins are transported to lysosome or endosome. A protein complex, coat protein complex II (COPII), is involved in this

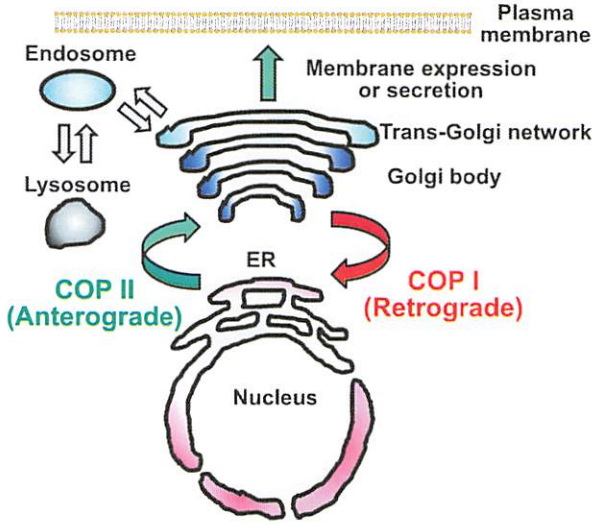

Fig. 6.4 Transport of intracellular proteins. COPII mediates anterograde transport from the ER to the Golgi, while COPI is involved in retrograde transport from the Golgi to the ER

transport from the ER to the Golgi, which is called as anterograde transport. Another protein complex, coat protein complex I (COPI), functions in the opposite transport from the Golgi to the ER, which is called as retrograde transport.

COPA syndrome was reported in 2015 as an autosomal dominant hereditary autoinflammatory disease characterized by interstitial pneumonia, arthritis, and glomerulonephritis [48]. The adaptive immunity such as T cells were also activated and helper T (Th) cell differentiation was skewed from Th1 to Th17 cells [1]. The responsible gene variants were identified as heterozygous missense variants in *COPA* encoding coatomer subunit alpha (COPA), a subunit of COPI. All variants are found in the WD40 domain, which is supposed to be involved in a protein–protein interaction (Fig. 6.5). COPA syndrome patients also manifest increased expression of IFN-stimulated genes (ISGs), i.e., type I interferonopathy. Recently, analysis on the mutant mice carrying the gene variants from COPA syndrome patients revealed the type I interferonopathy is caused by hyperactivation of the cytoplasmic DNA sensor signaling pathway [49, 50].

Matsubayashi et al. found four patients from one family with COPA syndrome-like symptoms such as interstitial pneumonia and arthritis and

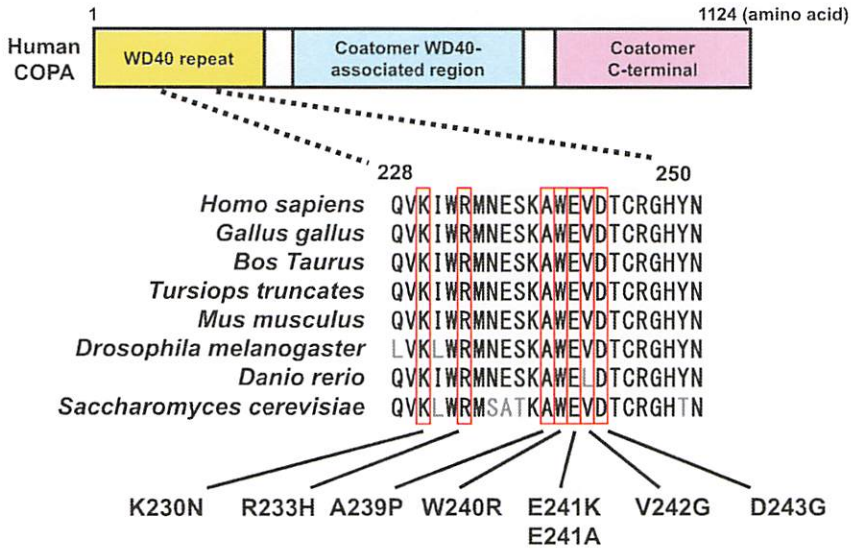

Fig. 6.5 Gene variants of the COPA syndrome patients. Gene variants of the COPA syndrome patients are found in the WD 40 domain of COPA

type I interferonopathy [50]. The whole exome analysis revealed a novel heterozygous missense variant (c.725 T > G, p.V242G) in *COPA* of the four patients. V242 is an amino acid conserved across species and is located in the WD40 repeat region, as found in COPA syndrome patients previously reported (Fig. 6.5).

6.7 COPA Syndrome Model Mice

We have then generated *Copa V242G* mice to clarify the pathological contribution of the variant [50]. Heterozygous *Copa V242G* mice appeared healthy at a glance, but showed histological abnormalities in their lungs from around 16 weeks after birth. The abnormalities include thickening of alveolar walls, infiltration of inflammatory cells including lymphocytes, and formation of lymph follicle-like structures, which are similar to pathological findings in the lungs of COPA syndrome patients. On the other hand, no histological abnormalities were observed in other organs such as joints, kidneys, or livers. In addition, expression of ISGs in the spleen was enhanced in heterozygous *Copa V242G* mice, compared to wild-type mice. Thus, the lung pathology and type I interferonopathy of COPA syndrome patients was recapitulated in the mutant mice. As for T cells, both CD4 and CD8 T cells in the spleen were normal in number in the mutant mice, but the percentage of naive T cells was decreased, while that of effector memory T cells was increased, indicating that T cells were significantly activated in the mutant mice. Concerning the cytokine production from T cells, IFN-γ-, rather than IL-17-producing cells were prominently increased in both CD4 and CD8 T cells from the mutant mice.

Representative sensors as type I IFN inducers are nucleic acid sensors. Those sensors include transmembrane sensors, Toll-like receptors (TLRs), TLR7 and TLR9, which sense single strand RNA and DNA carrying unmethylated CpG motif, respectively. In the cytosol, retinoic acid-inducible gene-I-like receptors (RLRs) and the cyclic GMP-AMP synthase (cGAS)-STING pathway functions as RNA and DNA sensors, respectively.

Among these sensors, the cGAS-STING pathway was supposed to be the candidate pathways upregulated in the *Copa V242G* mice. In the cGAS-STING pathway, cGAS senses double stranded DNA (dsDNA) and generates cyclic dinucleotides, cGAMP, which binds to STING in the ER (Fig. 6.6). Then STING forms a homodimer and is activated as it translocates to the Golgi, In the Golgi, STING activates a kinase, TANK binding kinase 1 (TBK1), which subsequently phosphorylates a transcription factor, interferon regulatory factor 3 (IRF3) and induce expression of type I IFNs and other cytokines. Thus, intracellular localization of STING is important in regulating the cGAS-STING pathway. Indeed,

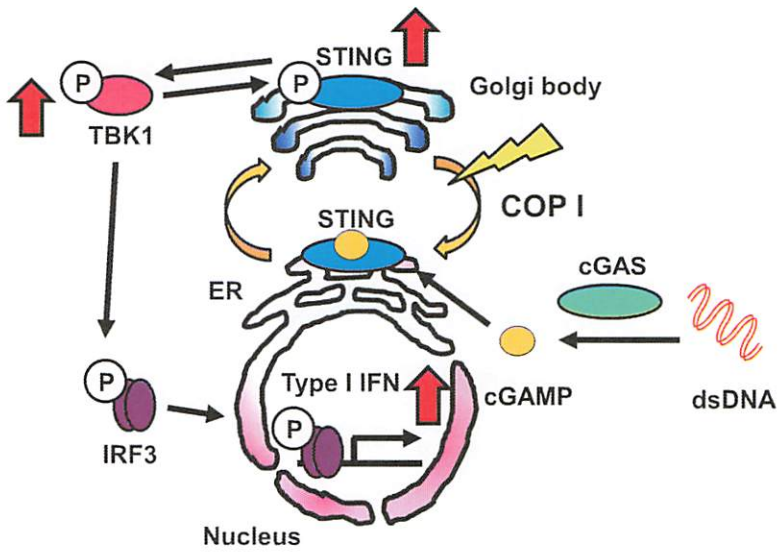

Fig. 6.6 Hyperactivation of the cGAS-STING pathway by the COPA syndrome gene variants. The COPA syndrome gene variants cause defect of COPI-mediated protein transport. As a result, STING shifts from the ER to the Golgi and subsequently activate the downstream pathway, leading to TBK1 activation and overproduction of type I IFNs

gene variants of STING in SAVI, which is characterized by interstitial lung disease and type I interferonopathy, render STING localized in the Golgi rather than the ER [17].

Then bone marrow-derived GM-CSF-induced cells consisting of DCs and macrophages revealed hyperactivation of the cGAS-STING pathway in the heterozygous *Copa V242G* mutant mice. The mutant cells produced more amounts of type I IFNs in response to a STING agonist. In resting states, TBK1 as well as STING were unphosphorylated and STING resided in the ER in mutant cells. However, upon stimulation with the STING agonist, phosphorylation of TBK1 and STING was more potently induced and STING tended to be localized in the Golgi, compared with wild-type cells. Meanwhile, type I IFN induction by the other nucleic acid sensors were rather decreased. Thus, *V242G* variant should cause defects in trafficking of STING and lead to accumulation in the Golgi and hyperactivation of the STING pathway (Fig. 6.6). Furthermore, STING inhibitors attenuated upregulated expression of ISGs in the spleen of the mutant mice, indicating that type I interferonopathy in the heterozygous *Copa V242G* mice is caused by upregulation of the cGAS-STING pathway.

Another group led by Drs. Shum and Taguchi also demonstrated activation of the cGAS-STING system due to another gene variant, E241K, of COPA syndrome by analyzing the cell lines and *Copa E241K* mice [49]. The heterozygous *Copa E241K* mutant mice showed not only interstitial pneumonia but also type I interferonopathy due to hyperactivation of the cGAS-STING pathway. Curiously, the mutant fibroblasts showed enhanced phosphorylation of TBK1 and STING and accumulation of STING in the Golgi prior to stimulation. It is currently unknown whether this phenotypic difference depends on the position of mutated amino acids or on the cell type analyzed. According to Taguchi, COPA does not directly bind to STING, but SURF4, a multi-pass transmembrane protein chaperone, mediates the interaction between COPA and STING [51]. Knockdown of subunits of COPI, including COPA, in THP-1 cells was also shown to cause hyperactivation of the cGAS-STING pathway [52]. Thus, COPA-mediated trafficking is critical for regulating the cGAS-STING pathway.

6.8 SAVI Model Mice

It should be useful to compare the phenotype of the COPA syndrome model mice with that os SAVI model mice. Several SAVI model mice have been generated and analyzed in several groups [53]. In the mutant mice carrying the *Sting N153S* and *Sting V154M*, STING is accumulated in the Golgi and the signaling pathway is upregulated. They also manifest interstitial pneumonia and type I interferonopathy, as observed in COPA syndrome model mice. However, the phe-

notype is variable, depending on the mutations or laboratories. For example, the heterozygous *Sting N153S* mice manifest intestinal inflammation with dysbiosis or skin ulcers [54, 55]. Kidney inflammation was reported in the heterozygous *Sting V154M* mice [56].

In the immune system, neutrophils and monocytes are increased, while lymphocytes are decreased in both *Sting N153S* and *V154M* mice. Thymus and lymph nodes are small, and T cells show enhanced cell death and their numbers are markedly decreased [55, 56]. B cells tend to be decreased, but in the heterozygous *Sting N153S* mice, serum IgM level is high and autoantibodies are detected [55].

Sting N153S mice have been crossed with several kinds of mutant mice. The analysis on the resultant mutant mice revealed that T cell deficiency abolished, but B cell or type I IFN receptor deficiency did not ameliorate the interstitial pneumonia [57]. The results suggest that T cells are critical, but B cells or type I IFN signaling are dispensable for development of interstitial pneumonia. Enhanced STING leads to hyperresponsiveness to T cell receptor-induced ER stress and disruption of calcium homeostasis, thereby inducing T cell death in a type I IFN-independent manner [58, 59]. It is, however, unknown how such T cell abnormality causes interstitial pneumonia. In addition to the direct consequence of STING signaling activation, it is also possible that abnormal T cell activation secondary to enhanced cell death is involved.

Both SAVI and COPA model mice have in common the development of interstitial pneumonia, increased ISGs, accumulation of STINGs in the Golgi, and T cell activation. Interstitial pneumonia in the heterogenous *Copa E241K* mice is dependent on T cells [60]. Meanwhile, T cells in the mutant mice are activated, but does not manifest enhanced cell death and their numbers are not decreased. It should be important to clarify how T cells are involved in the development of interstitial pneumonia of COPA model mice.

In addition, there are still many important issues to be resolved in the pathogenesis of COPA syndrome. It is unknown which pathway is responsible for the pathogenesis, type I IFN induction or inflammatory cytokine induction pathway, or both. Furthermore, it is still possible that any other molecules in addition to STING, transport of which is impaired, contribute to the pathogenesis.

6.9 Summary

During these past 20 years identification of human IEIs has greatly advanced. Furthermore, it is also important to note that progress of genome editing techniques, especially the CRISPR/Cas9 technology, has allowed us to easily introduce the gene variants and generate the mutant mice carrying them.

Each monogenic disease itself is very rare, but similar or related dysfunctions can also be seen in more common diseases. For example, inflammasome activation is observed not only in autoinflammatory diseases but also in gouty arthritis or atherosclerosis. Concerning the proteasome, its activity is upregulated in cancer cells and proteasome inhibitors are used for treating multiple myeloma. The proteasome dysfunctions are also observed in a variety of pathological conditions including aging, neurodegenerative diseases, or intestinal bowel diseases. Concerning the STING pathway, aberrant or ectopic accumulation of DNAs leads to the activation of the pathway, which plays a driving role in progression of a variety of inflammatory or degenerative diseases [61]. They include neurological disorders such as amyotrophic lateral sclerosis and Parkinson disease, autoimmune diseases such as SLE, and metabolic diseases such as nonalcoholic steatohepatitis. Some antitumor therapies were also found to depend on the cGAS-STING pathway. Furthermore, interstitial pneumonia and type I interferonopathy are also observed in polygenic autoimmune diseases such as SLE. Thus, elucidation of the pathogenesis of monogenic diseases should pave the way for understanding and manipulating the pathological conditions in common polygenic diseases. Furthermore, unexpected link between the mutated molecules and the manifestations should also lead to clarifica-

tion of novel regulatory mechanisms for immune responses.

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