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Mini Review

Sarcoidosis and Autoinflammation

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Sporadic early-onset sarcoidosis (EOS) and familial Blau syndrome (BS) form a distinct set of autoinflammatory diseases, both of which onset in infancy and show a clinical triad of dermatitis, arthritis and uveitis histologically composed of noncaseating epithelioid cell granuloma. The responsible gene for EOS/BS is *NOD2*, encoding an intracellular receptor for muramyl dipeptide (MDP), the common component of bacterial cell wall peptidoglycan. The gain-of-function *NOD2* mutations with MDP-independent basal NF-κB activation cause EOS/BS, while its loss-of-function mutations with impaired MDP-dependent NF-κB activation are associated with Crohn's disease and a part of sarcoidosis. As the result of analyzing genotype-phenotype relationship of Japanese EOS/BS cases, somewhat positive correlation was recognized between mutant *NOD2*-causing basal NF-κB activation and clinical severity, especially of ocular complications. Role of the basal NF-κB activation in monocytes has also been suggested by an inhibitory effect of thalidomide on *ex vivo* giant cell formation from EOS/BS patients' monocytes. Analysis of mutant *NOD2*-introduced human monocytic THP-1 cells, as well as the patients' samples, would provide further detailed molecular mechanisms of EOS/BS pathogenesis, in which a single point missense mutation causes a distinct pathological change forming epithelioid cell granuloma.

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Early-onset Sarcoidosis (EOS) and Blau Syndrome (BS)

Sarcoidosis is a multiorganic inflammatory disease with unknown etiology, characterized by the histologic features of noncaseating epithelioid cell granuloma. It was already known that there is a rare but distinct type of sarcoidosis called EOS (MIM#609464), characterized by the onset in infancy and a triad of arthritis, uveitis and skin disorder, without apparent involvement of lung and hilar lymph nodes (Fig. 1)[1]. In contrast with ordinary sarcoidosis with a triad of lung disorder, lymphadenopathy and uveitis, which can be accidentally found by routine chest roentgenogram in elder children and adults, EOS typically starts with skin eruptions before 4 years of age and develops arthritis and uveitis in this order, not accompanying abnormalities on chest roentgenogram. It should be noted that, without histological examination, EOS patients can be easily misdiagnosed as suffering from juvenile idiopathic arthritis (JIA) because of their remarkable arthritis[2]. However, arthritis in EOS starts with synovial cyst without pain or abnormal change on roentgenogram and therefore should be distinguished from JIA. Total uveitis is characteristic in EOS and also can be distinguished from anterior uveitis observed in JIA. Skin eruptions are also characteristic with multiple lichenoid maculopapules showing tapioca-like appearance, which are rarely seen in ordinary sarcoidosis and classified into the lichenoid-type according to the Scadding's classification of skin sarcoidosis[3]. Although only 20 or less EOS cases have been reported in Japan, many cases are progressive and some of them finally show blindness, joint contracture with camptodactyly and visceral involvement[2].

In 1985, a large family in 4 generations showing EOS-like systemic granulomatosis with a triad of arthritis, uveitis and skin rash was reported by Edward B. Blau and a new entity, BS (MIM#186580), has been designated[4]. Actually, it has been discussed, whether BS and EOS are the same or not, since the first report by Blau[5]. However, BS has been defined as a distinct disease from EOS, a subtype of sarcoidosis, by its apparent autosomal dominant inheritance. Histologically, it is hard to distinguish these diseases with each other and from ordinary sarcoidosis. Their clinical features are also undistinguishable with each other but clearly different from those of sarcoidosis.

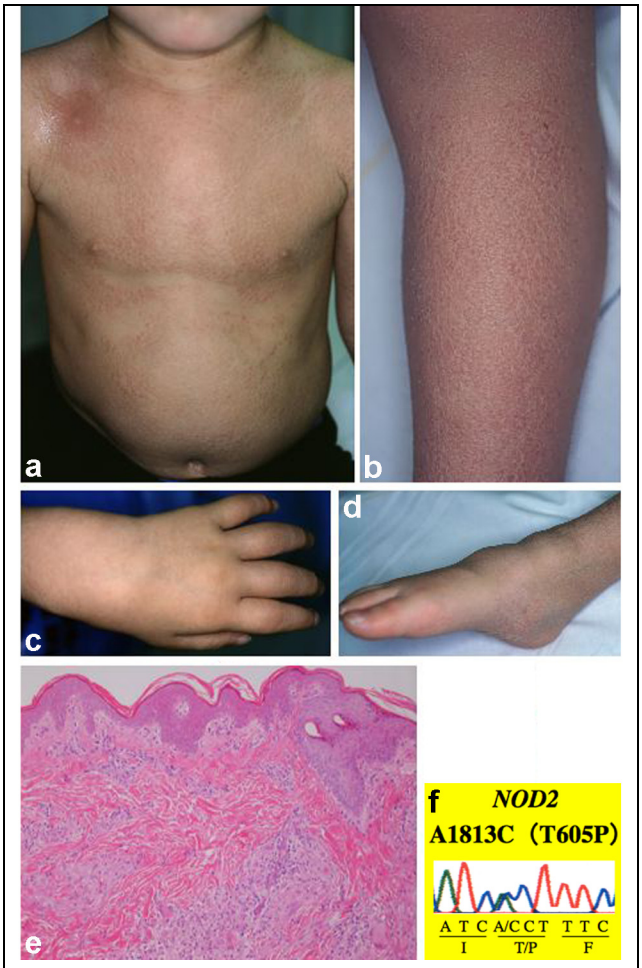

Figure 1. A 3-year-old boy with early-onset sarcoidosis.

(a-d) Gross appearance. Typical multiple lichenoid papules in the trunk

(a) and the arm (b) and cystic swelling of the wrist (c) and the ankle (d) joints are shown.

(e) Histological manifestation by the Hematoxylin and Eosin stain. Naked epithelioid cell granulomas with a multinucleated giant cell are apparent.

(f) NOD2 mutation. Heterozygous 1813A>C mutation causing T605P amino acid transition was identified.

NOD2 mutations responsible for EOS/BS

By linkage analysis, the responsible gene for BS was mapped on chromosome 16, close to the *IBD1* locus which was predicted to be associated with Crohn's disease (CD), a granuloma-forming inflammatory bowel disease[6]. Soon after the *NOD2 (CARD15)* gene

was identified to be responsible for CD in 2001[7,8], mutations of the same gene were searched in familial BS cases and novel heterozygous mutations were identified[9]. In this report, no similar mutations were detected in sporadic EOS cases and therefore different etiology of EOS and BS was proposed. In 2004 and 2005, we identified heterozygous mutations of the *NOD2* gene in most Japanese EOS cases, some of which had already been found in BS, and suggested the same etiology of these diseases[10,11]. Although no association of CD-related mutations has been identified in Japanese CD cases, EOS/BS-causing mutations were reported in both Caucasians and Asians and the new designation "pediatric granulomatous arthritis" has been proposed to unify them in the international registry[12].

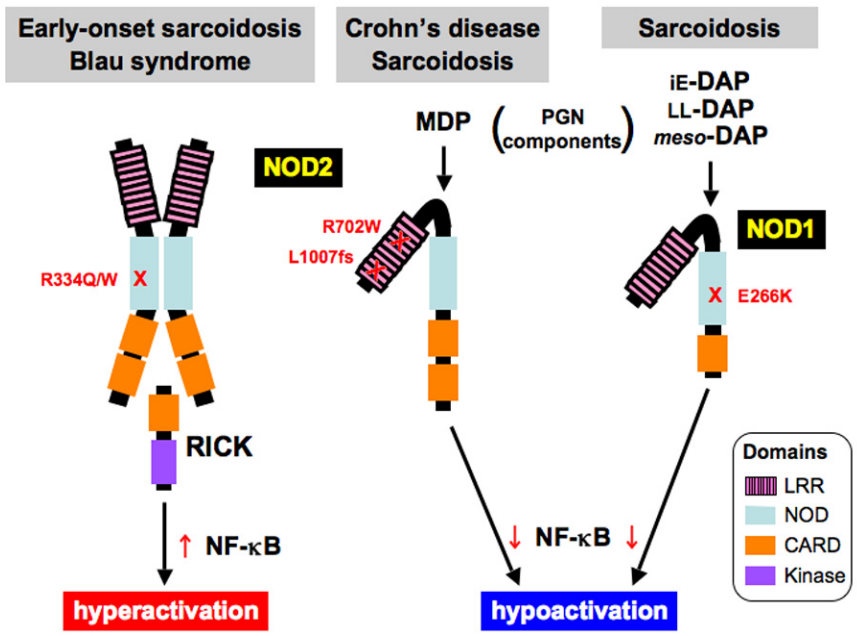

Figure 2. Genetic backgrounds of granuloma-forming diseases.

PGN: ppeptidoglycan, DAP: diaminopimelic acid.

NOD2 is expressed in cytoplasm of monocytic cells and epithelial cells and composed of 3 domains, caspase recruitment domain (CARD), nucleotide-binding oligomelization domain (NOD) and leucine rich repeats (LRR) (Fig. 2). In the steady state, the NOD2 molecule is folded and the region of LRR covers the NOD to inhibit its dimerization-induced activation. Upon recognition through the LRR of muramyl dipeptide (MDP), the minimum and common immunocompetent module of both gram-negative and positive bacterial cell wall peptidoglycan, the NOD2 molecule is unfolded to dimerize through the NOD and interacts through the CARD with a serine threonine kinase RICK (receptor-interacting protein-like interacting CLARP (caspase-like apoptosis-regulatory protein) kinase). Subsequently ubiquitinated RICK activates nuclear factor (NF)-κB through interaction with complexes of TGFβ-activated kinase 1 (TAK1) and inhibitor of NF-κB kinase (IKK)[13]. While loss-of-function mutations in the LRR, by which MDP-dependent NF-κB activation is impaired, are reportedly associated with CD, gain-of-function mutations in the NOD, causing MDP-independent constitutive NF-κB activation, is associated with EOS/BS. Additionally, a loss-of-function mutation in the NOD of the NOD1 gene, by which intracellular recognition of Propionibacterium acnes was impaired, is reportedly associated with ordinary sarcoidosis in the Japanese[14]. More recently, one of the CD-associated NOD2 mutations has been reported to be associated

with severe pulmonary sarcoidosis[15]. Collectively, as genetic backgrounds of granuloma-forming diseases, CD and sarcoidosis are associated with the NOD1/2 mutations causing hypoactivation to bacterial components, while EOS/BS are caused by the NOD2 mutations leading to constitutive hyperactivation (Fig. 2). Consistent with these results, presence of MDP was histologically revealed in sarcoid granuloma[16]. In 2010, another line of evidence has been added for the role of the NOD2 signaling on bacterial sensing and granuloma formation, since a genomewise association study in China has shown an association of both the NOD2 and the RICK genes with Hansen's disease, a granuloma-forming chronic infectious disease caused by low virulent bacilli, Mycobacterium leprae[17].

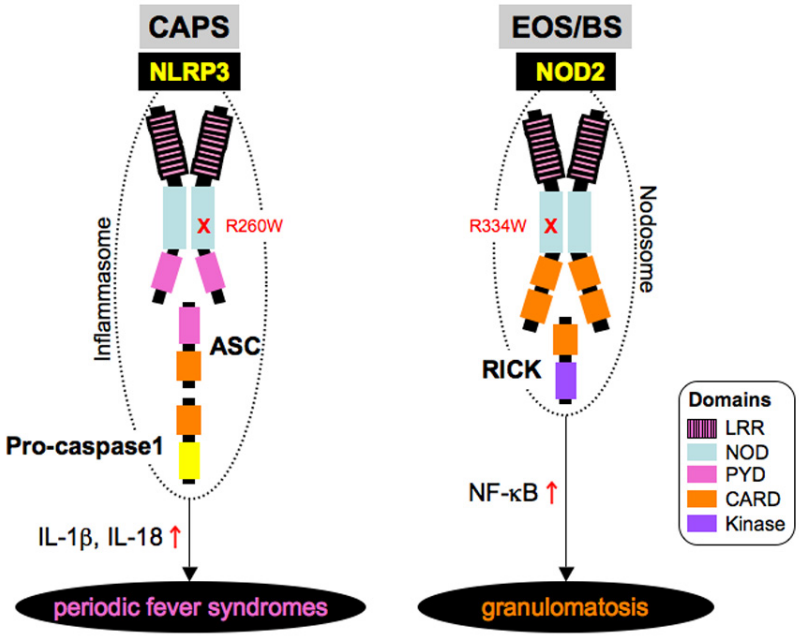

Figure 3. Comparison of NLRP3 and NOD2 signaling.

ASC: apoptosis-associated speck-like protein, PYD; pyrin domain.

NOD2 shares the common NOD-LRR domain structure with NLRP molecules which contains the pyrin domain (PYD) instead of the CARD. They form NLR family molecules and NOD2 (CARD15) is formally renamed as NLRC2[18]. Interestingly, the most frequent EOS/BS-associated mutations in NOD2 / NLRC2, R334W/Q, correspond to the CIAS1 / NLRP3 mutations, R260W/Q, which are associated with the major autoinflammatory disease, cryopyrin-associated periodic syndrome (CAPS) (Fig. 3)[19]. In contrast with the NLRP3 inflammasome, the molecular complex of NOD2 and RICK is called as "nodosome"[20]. So far 3 other NLR genes have been identified in hereditary autoinflammatory / autoimmune diseases, NLRP1 in vitiligo-associated multiple autoimmune / autoinflammatory diseases, NLRP7 in recurrent hydatidiform moles and NLRP12 in familial cold-induced autoinflammatory disease (FCAS) 2[20,21].

Role of constitutive NF-κB activation in granuloma formation

To confirm the role of constitutive NF-κB activation in mutant NOD2-associated granuloma formation, 20 Japanese EOS/BS (14 EOS and 6 BS) cases with NOD2 mutations were collected and their genotype-phenotype correlation was analyzed[22]. For an indicator of a mutation-induced cellular abnormality, NF-κB activity of a mutant NOD2-transfected HEK293 cells in the absence and the presence of MDP was measured with the luciferase assay and the ratio of basal NF-κB

activation in the absence of MDP to that in the presence was calculated. In contrast with 0.05 in case of the wild-type NOD2, the ratio ranged from 0.2 in E383G to 0.8 in C495Y mutation. In Table 1, genotype and phenotypic features of the 20 cases were listed in the order of this basal NF-κB activation ratio. Age of the onset of each phenotype tends to be younger as the ratio of basal NF-κB activation is higher, excluding many exceptional cases. Concentrated on ocular complication in 3 cases with R334Q and 9 cases with R334W, all R334Q cases show normal visual acuity while 4 cases with R334W suffer mild to severe impairment of visual acuity, in correlation with the basal NF-κB activation ratio, which is higher in R334W than in R334Q. This result suggests that determination of the NOD2 genotype predicts the clinical outcome of EOS/BS.

Furthermore, a recent report showing effectiveness of an NF-κB inhibitor thalidomide on EOS/BS cases added an another line of evidence for the role of constitutive NF-κB activation on progression of the diseases[23]. In this report, multinucleated giant cells were specifically formed from a patient's peripheral blood monocytes with macrophage colony-stimulating factor (M-CSF) and interleukin (IL)-4 and thalidomide showed an inhibitory effect on this ex vivo giant cell formation, suggesting the role of constitutive NF-κB activation in monocytes on granuloma formation. Then, how epithelioid cell granuloma is generated by such constitutive NF-κB activation in monocytes? To address this issue, we have generated human monocytic THP-1 cells transfected with EOS/BS-associated mutant NOD2 and analyzed their phenotypic abnormalities. Unexpectedly, no change was observed between the wild-type and mutant cells on levels of cytokine productions in the absence or even in the presence of MDP and/or TLR ligands. Instead, after addition of phorbol myristate acetate (PMA), which allows THP-1 cells to differentiate into macrophage-like adherent cells, mutant cells specifically attached to the culture plate for a long period with sustained expression of surface intercellular adhesion molecule (ICAM)-1 and transiently express platelet-derived growth factor (PDGF)-B, not IL-8 or tumor necrosis factor (TNF)α. As coexpression of NOD2 with ICAM-1 and PDGF-B was revealed in multinucleated giant cells forming the granulomatous skin lesion of an EOS patient, involvement of these molecules in mutant NOD2-associated granuloma formation was suggested (Nishiyama et al: manuscript in preparation). Considering these results, peripheral blood monocytes in EOS/BS patients possibly express sustained surface ICAM-1 and produce an abnormally high amount of PDGF-B when recruited to the lesional tissue and differentiate into macrophages in response to some differentiation signal. Such biological changes would contribute to the "automatic" formation of epithelioid cell granuloma. Although experimental evidence is still absent, T cells might be unnecessary for this EOS/BS-type NOD2 mutation-causing granuloma formation, similar to the mice targeted with CAPS-associated NLRP3 mutations[24].

Concluding remarks

Although CD has been the most investigated disease among NOD2-associated autoinflammatory diseases, EOS/BS are those caused by the gain-of-function mutations of an NLR family gene, which should be corresponding to CAPS. It is expected that further investigation of EOS/BS, in which only a single missense mutation causes a distinct pathological change of epithelioid cell granuloma, elucidates the molecular mechanisms of a unique chronic inflammation with granuloma formation and promotes the development of more effective molecular target drugs.

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