



Mini Review

The roles of NOD like receptors in inflammation are different between Japanese and Caucasian.

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Nucleotide binding and oligomerization domain (NOD)-like receptors (NLRs) are members of the innate immune system that recognize pathogens in the cytosol. We have previously revealed that multiple genetic variants of NOD1 and NOD2, components of NLRs, are associated with susceptibility to several granulomatous diseases. Notably, NOD2 loss-of-function and gain-of-function mutations showed susceptibility to Crohn's disease and Blau syndrome, respectively. Furthermore, we have revealed that impaired recognition of intracellular *Propionibacterium acnes* resulting from a polymorphism in the NOD1 gene is involved in the increased susceptibility of Sarcoidosis in a Japanese population. In this review, we examine the function of NOD1 and NOD2 in innate immunity, with a focus on their differing roles in disease pathogenesis between Japanese and Caucasian populations.

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Introduction

In the recognition of and reaction to pathogens, hosts utilize two types of immune mechanisms, adaptive and innate system, to effectively eliminate the pathogens. Fast innate immune responses are mediated by a set of non-clonal, germline-encoded pattern-recognition receptors (PRRs) that react against conserved structures in pathogens, named pathogen-associated molecular patterns (PAMPs). PAMPs include lipopolysaccharides, peptidoglycan, unmethylated CpG DNA, and endogenous danger signals including uric acid and heat shock proteins (HSP). Various PRRs exist in the extracellular space and in the cytosol.

PRRs mainly consist of two groups, toll-like receptors (TLRs) and nucleotide binding oligomerization domain (NOD)-like receptors (NLR) (Fig.1). TLRs represent a class of membrane-bound PRRs that recognize a variety of PAMPs at the cell surface and within endosomes¹⁾. NLRs consist of cytoplasmic PRRs that play an essential role in recognizing PAMPs in the cytosol²⁾. The NLRs consists of about 20 proteins that are characterized by the presence of a conserved tripartite domain structure (Fig.2): a central NOD domain that takes roles in self-oligomerization, C-terminal leucine-rich repeats (LRRs) that are involved in PAMPs recognition, and an

N-terminal effector-binding domain, such as the Pyrin domain (PYD) and the caspase recruitment domain (CARD)²⁾.

NOD1 and NOD2

In early studies, NOD1 and NOD2, members of NLRs, were found to activate NF- κ B in mammalian cells in response to pathogens independently of TLRs³⁾. Subsequent studies showed that NOD1 recognizes meso-diaminopimelic acid (iE-DAP), which is found in many Gram-negative and certain Gram-positive bacteria⁴⁾. NOD2 recognizes muramyl dipeptide (MDP), a component of peptidoglycan that is present in both Gram-positive and -negative bacteria⁵⁾. Fig.2 shows the reaction cascade of NOD2. Nod2 undergoes conformational changes upon binding of ligands (Step 1), resulting in self-oligomerization via the central NOD domain (Step 2) and recruiting to RICK (RIP2), a serine threonine kinase that is indispensable for NF- κ B activation⁶⁾ (Step 3). The tissue distributions of NOD1 and NOD2 are quite different. Nod1 is ubiquitously expressed in various tissues and Nod2 is expressed in the Paneth cells of the small intestine and monocytes²⁾. *In vivo* studies have revealed that NOD1 ligands simulate chemokine production and recruitment of neutrophils⁷⁾, and contribute to adaptive immune responses.

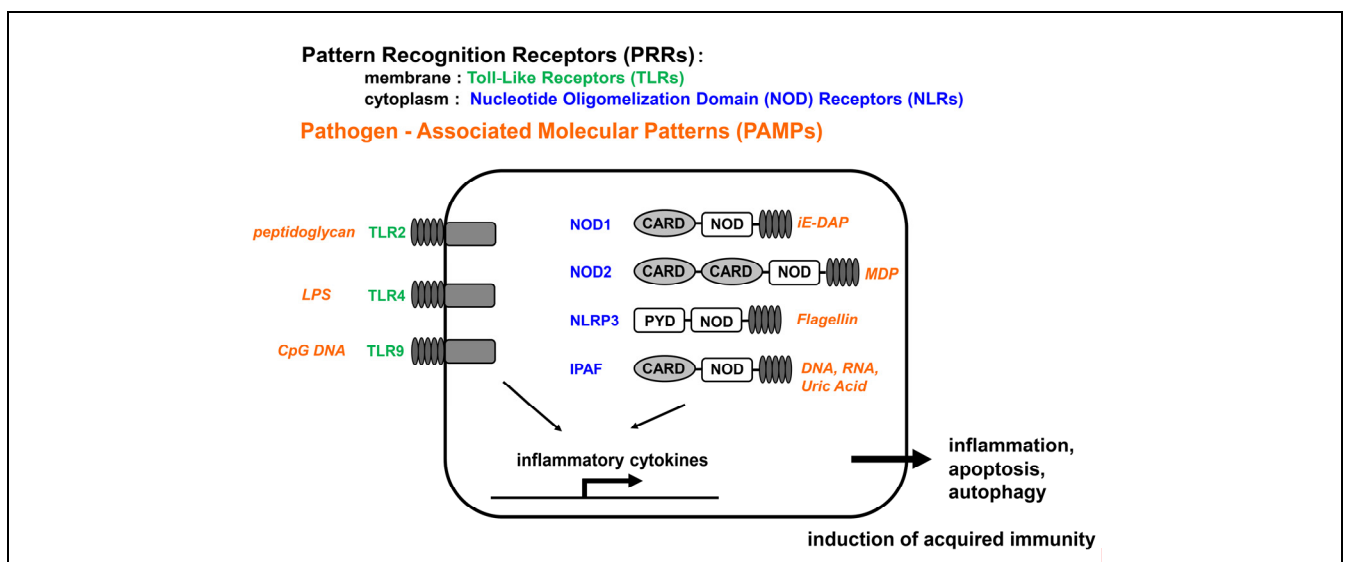
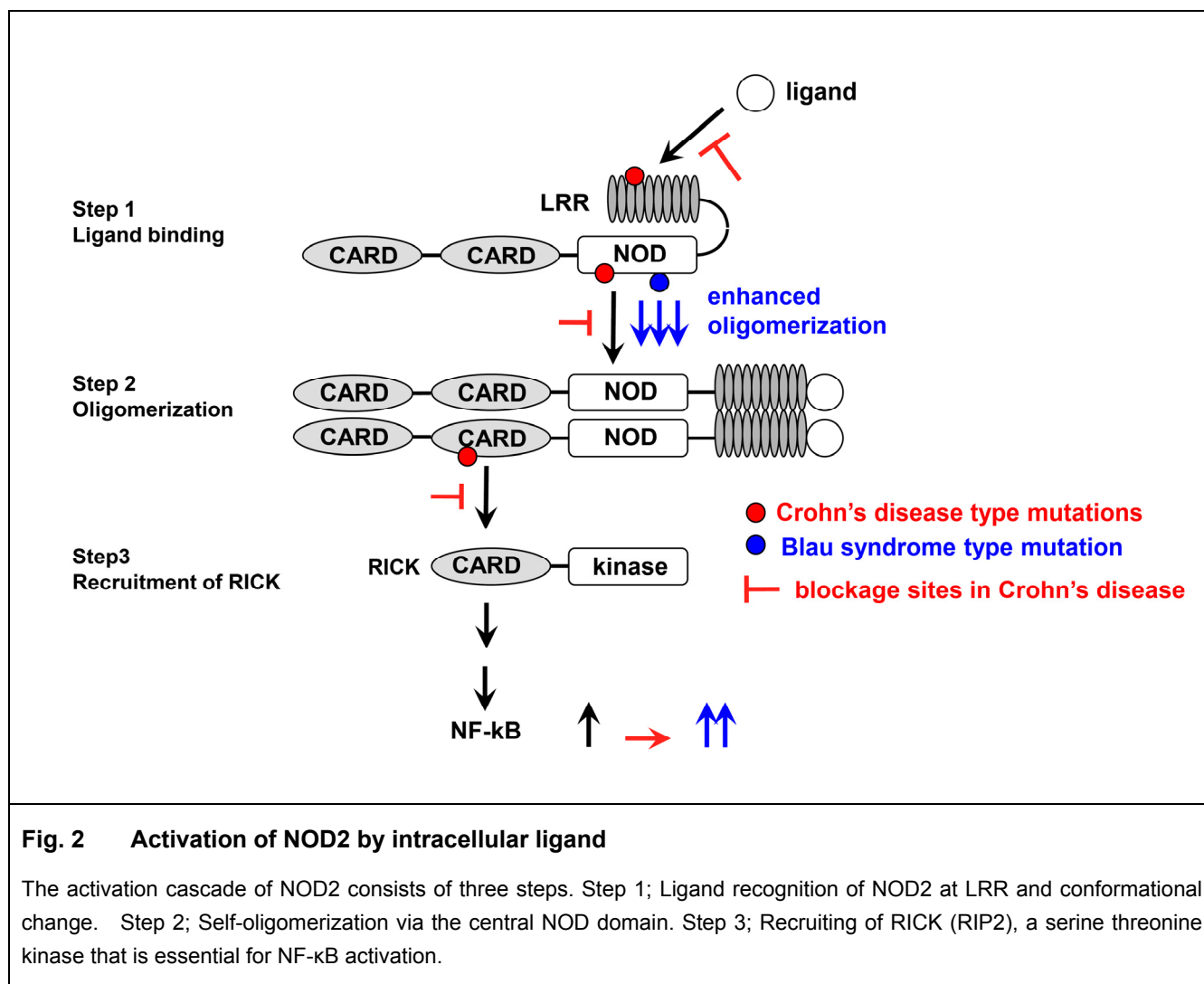


Fig. 1 Models for PAMPs recognition by NLRs and TLRs

The innate immune system consists of PRRs including NLRs and TLRs. NLRs and TLRs sense intracellular and extracellular PAMPs, respectively. The subsequent activation of inflammatory cytokines results in the inflammation, apoptosis, and autophagy. Both pathways involved in the induction of acquired immunity.



Crohn's disease

Crohn's disease (CD) is a chronic inflammatory bowel disease. The association between genetic polymorphism of NOD2 and susceptibility to Crohn's disease has been reported in Caucasians⁸. The genetic variants of NOD2, which include three common mutations (R702W, G908R, L1007insC) that involve amino acid residues near or within the LRRs of Nod2, are associated with the development of CD (Fig.3). Ethnic differences in the genetic susceptibility to Crohn's disease have been shown between Caucasian and Japanese populations. Some studies have revealed that any of the three common NOD2 mutations which have been found in CD patients in Caucasians are absent in Japanese CD patients⁹. Also, sequencing analysis of the all exons of NOD2 in Japanese CD patients reported no common genetic variants¹⁰. These results indicate that, unlike in

Caucasians, the *NOD2* gene is not a major contributor to CD susceptibility in the Japanese population.

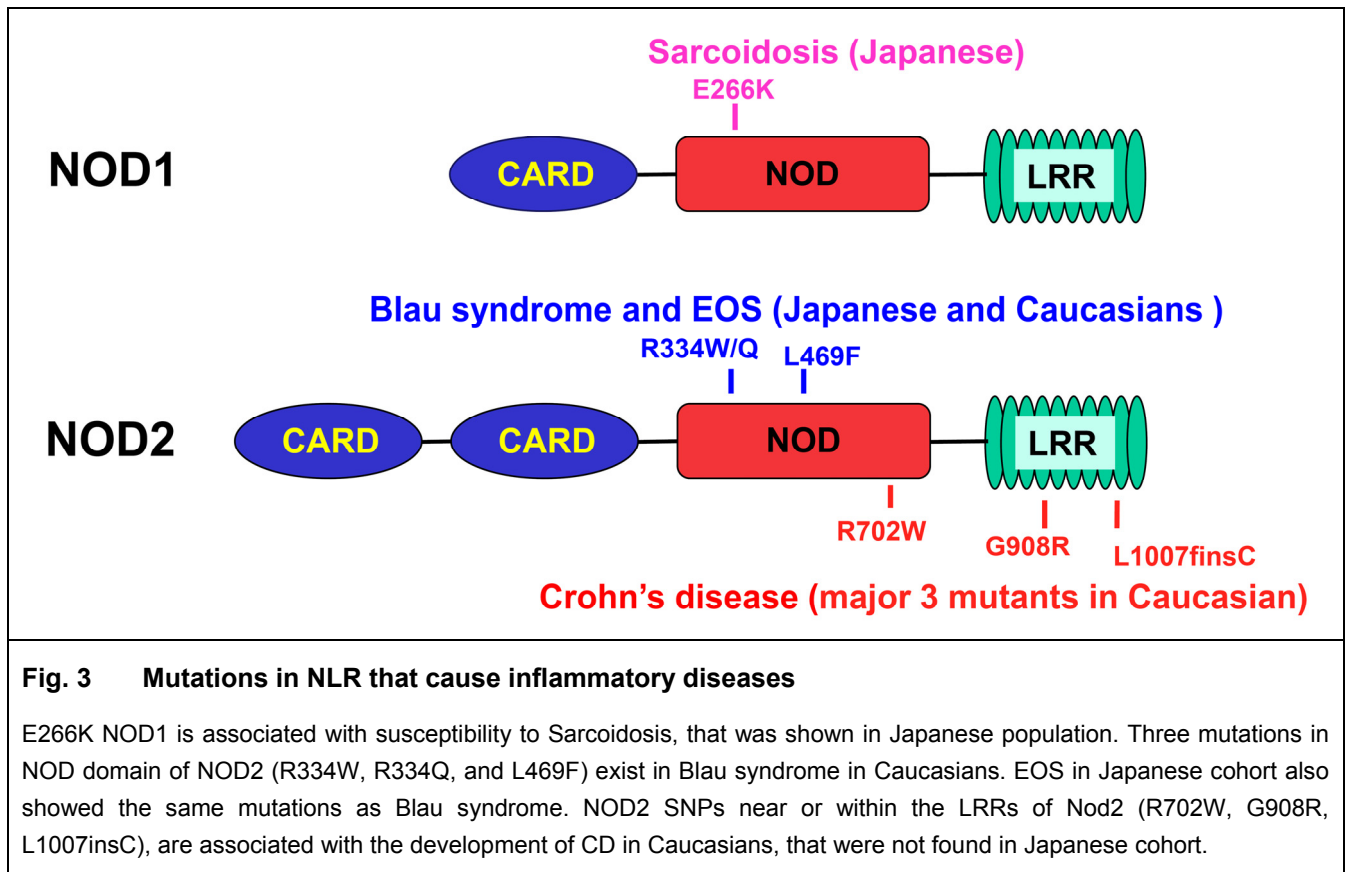
CD-associated mutations inhibit either of the three steps including conformational change after ligand recognition, self-oligomerization, and recruitment of RICK (Fig.3), leading to the reduction or loss of NF-κB activation upon MDP-stimulation, compared with the wild-type protein² (Fig.2).

The mechanism by which Nod2 mutations increase the susceptibility to CD remains controversial. Nod2-deficient mice provide a possible mechanism of Nod2 mutations involved in susceptibility of CD¹¹. These models show that the Nod2 protein is a key regulator of infectious immunity within the intestine. Further, protective immunity, mediated by Nod2 recognition of bacterial muramyl dipeptide, is abolished in Nod2-deficient mice. These animals were susceptible to bacterial infection via the oral delivery, but not through intravenous or peritoneal route, and Nod2 was necessary for the expression of one of in-



testinal anti-microbial peptides, known as defensins. The impaired or loss of function of NOD2 may induce a complex defect of innate immune system leading to facilitated entry of bacteria into epithelial cells through defective expression of defensin, resulting in an abnormal inflammatory response to invaded bacteria.

An alternative hypothesis has recently been shown through the analysis of a transgenic murine model that carries a NOD2 variant equivalent to the human L1007insC mutation. Intriguingly, mouse macrophages, but not human monocytes, that express the CD-associated L1007insC NOD2 variant exhibit increased IL-1 β levels when stimulated with MDP¹²⁾.



Blau syndrome

Blau syndrome (BS) is a rare autosomal dominant disorder characterized by early-onset granulomatous inflammation including arthritis, uveitis, and dermatitis with camptodactyly¹³⁾. Nod2 mutations were investigated in families with BS¹⁴⁾ because BS susceptibility locus has been identified at same locus to which NOD2 also has been mapped. BS families shared three missense mutations (R334Q, L469F and R334W) (Fig.3) in NOD domain. These mutations enhance the self-oligomerization of NOD2 (Fig.2) leading to the augmented NOD2 activity, even in the absence of the ligand MDP, and further increase the activity by the addition of ligands¹⁵⁾ (Fig.4), representing gain-of-function mutations, which is consistent with the dominant mode of inheritance of the granulomatous disease.

Early-onset sarcoidosis (EOS) is a systemic granulomatous syndrome sharing the distinct triad of skin, joint, and eye inflammation with BS, and is progressive and causes severe complications, such as destructive arthropathy and blindness. Interestingly, the majority of the EOS cases analyzed in Japanese cohort also showed the same mutations as Blau syndrome¹⁶⁾. Thus, EOS shares a common genetic etiology of Nod2 with Blau syndrome.

Sarcoidosis

Sarcoidosis, a systemic granulomatous disease of unknown etiology, presents symptoms including bilateral hilar adenopathy, granuloma formation in lung and eye. Sarcoidosis may result from the exposure of genetically susceptible subjects to specific agents, possibly bacteria. *Propionibacterium acnes* (*P. acnes*) is the only bacterium to be isolated from



affected lesions¹⁷⁾. Especially, intracellular type of *P. acnes* was prevalent in sarcoid lesions. Our analysis in the Japanese population revealed that NOD1 E226K variant associated with susceptibility to Sarcoidosis (Fig.3) and that the activity of the E226K SNP to induce NF- κ B production in response to intracellular *P.acnes* was impaired for about 50% compared with that of wild type (Fig.4). Genetic screening of the NOD2 gene in sarcoidosis revealed no common mutations both in Japanese and Caucasian subjects^{19, 20)}.

Conclusions

NLR-related diseases have been shown to have ethnic differences, between Caucasians and Asians. Susceptibility to Crohn's disease showed a correlation with NOD2 mutations in Caucasian, but not in Japanese, populations. This difference may be primarily due to the fact that three major NOD2 SNPs that are prevalent in Caucasians being absent in Japanese populations. Because NLR exerts widespread effects on cytokine secretion, cell survival, autophagy, and apoptosis, it is possible that many as yet uncharacterized diseases will also show different susceptibilities between races.

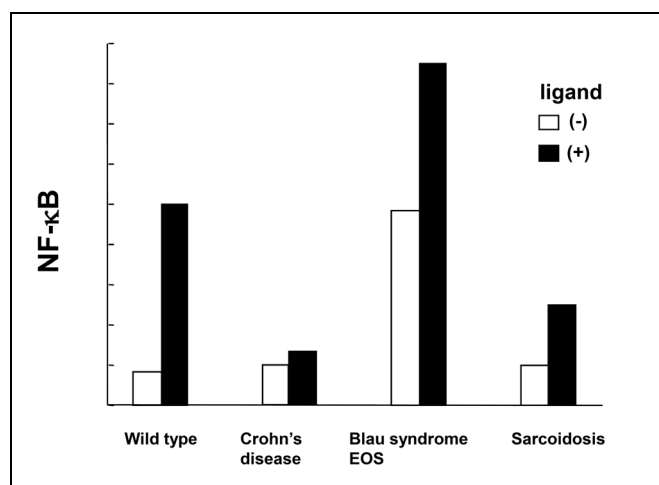


Fig. 4 Schematic activation pattern in NLRs related diseases

The production of NF- κ B is increased in response to ligand. Crohn's disease mutants lose or decrease the response. The mutations in Blau syndrome and EOS cause elevated NF- κ B production even in the absence of ligand, and further elevated the activity by the addition of ligand. Sarcoidosis type NOD1 SNP caused reduced activity for about 50% compared with that of wild type.

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