VIEW POINT

Plant Signaling & Behavior 9, e28197; March; © 2014 Landes Bioscience

Immunosuppression during *Rhizobium*-legume symbiosis

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Rhizobium infects host legumes to elicit new plant organs, nodules where dinitrogen is fixed as ammonia that can be directly utilized by plants. The nodulation factor (NF) produced by *Rhizobium* is one of the determinant signals for rhizobial infection and nodule development. Recently, it was found to suppress the innate immunity on host and nonhost plants as well as its analogs, chitins. Therefore, NF can be recognized as a microbe/pathogen-associated molecular pattern (M/PAMP) like chitin to induce the M/PAMP triggered susceptibility (M/PTS) of host plants to rhizobia. Whether the NF signaling pathway is directly associated with the innate immunity is not clear till now. In fact, other MAMPs such as lipopolysaccharide (LPS), exopolysaccharide (EPS) and cyclic-β-glucan, together with type III secretion system (T3SS) effectors are also required for rhizobial infection or survival in leguminous nodule cells. Interestingly, most of them play similarly negative roles in the innate immunity of host plants, though their signaling is not completely elucidated. Taken together, we believe that the local immunosuppression on host plants induced by *Rhizobium* is essential for the establishment of their symbiosis.

Keywords: nodulation factor, *Rhizobium*, legume, symbiosis, PAMP triggered susceptibility, immunosuppression

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Submitted: 01/11/2014

Revised: 02/12/2014;

Accepted: 02/12/2014

Published Online: 03/24/2014

Citation: Luo L, Lu D. Immunosuppression during Rhizobium-legume symbiosis. Plant Signaling & Behavior 2014; 9:e28197; PMID: 24556951; http://dx.doi.org/10.4161/psb.28197

During microbe-plant interactions, the microbe/pathogen associated molecular pattern (M/PAMP, including fragments of flagellin and EF-Tu) produced by microbes, triggers the innate immunity of host plants (PTI).[1] It is the first line of plants combating with microbes. To break this line, many bacteria inject several effectors into host cells through a type-III secretion system (T3SS) to disturb PTI for triggering susceptibility (ETS), and successfully invade hosts.[2] However, plants evolve a serial of resistance (R) proteins to recognize those effectors for induction of hypersensitive response (HR) and cell death to prevent further invasion, called effectors triggered immunity (ETI).[2] Noticeably, M/PAMPs can also trigger susceptibility (M/PTS) of a host to microbes or pathogens. For example, cyclic-β-glucan from *Xanthomonas campestris* pv *campestris* suppresses host immunity and enhances bacterial infection.[3] Compared known data, we find that M/PTS and ETS share similar features: (1) both types of immune reagents produced by microbes, (2) suppressing host immune or defense response to promote infection. Therefore, both M/PTS and ETS are immunosuppression responses, which have been described in mammalian immunology.

Symbiosis is established between *Rhizobium* and legumes through complex mutual interactions. During this process, rhizobia infect host plants through a channel (thread) on the tip of a root hair cell or a crack between two plant cells. Then the duplicated bacterial cells release from infection threads into plant nodule cells via endocytosis. After proliferation and differentiation, they turn into bacteroids fixing dinitrogen as ammonia. Several signals from rhizobia have been identified including nodulation factors (NFs), lipopolysaccharide (LPS) and exopolysaccharide (EPS).[4,5] Here, we re-examine these molecules and their signaling at the point of immunology.

NF is a group of lipo-chitin oligosaccharide synthesized by most rhizobia after treatment by specific flavonoids from host legumes.[6] NF is required for rhizobial infection and nodule development for most *Rhizobium*-legume symbioses.[6] NF works as a signal perceived by a couple of LysM receptor kinases (including LjNFR1–5, MtNFP and MtLYK3) to elicit calcium spiking and reprogram the expression of downstream genes.[7] Several genes (such as SYMRK, CCAMK, NIN, NSP1 and NSP2 in *Lotus japonicus*) have been identified to consist of a NF signaling pathway.[7] It has been reported that NF plays its regulatory roles in reactive oxygen species (ROS) production.[8,9] Interestingly, the MAPK cascade is associated with NF signal transduction.[10] Therefore, according to composite elements, the NF signaling pathway is similar with those PTI signaling pathways. Noticeably, *Bradyrhizobium japonicum* NF has been found to suppress the immune response on the leaves of nonhost *Arabidopsis thaliana* and host soybean.[11] Although GmNFR1 and GmNFR5 were not essential for this immunosuppression in leaves and a new LysM kinase could perceive NF in *Arabidopsis*, it is possible that the NF signaling pathway is associated with suppression of host defense in legume roots.

LPS is composed of core oligosaccharide, lipid A and O-antigen as a component of cell wall in Gram negative bacteria including all *Rhizobium* species.[12] LPS produced by some pathogens acts as a pathogenic factor.[13] Biosynthesis of LPS is essential for *Rhizobium* infecting host plants or survival in host cells.[14] It is interesting that purified LPS from *Sinorhizobium meliloti* can suppress the oxidative burst in *Medicago truncatula* suspension cells, but elicit it on non-host plants.[15] These data suggest that rhizobial LPS plays key roles in local immunosuppression of host legumes during symbiosis. Although the signal transduction of LPS is not clear in plants, it is possible that LPS negatively modulates host immunity during most of *Rhizobium*-legume symbioses.

Almost all rhizobia can produce at least one type of EPS.[16] *S. meliloti* produces two types of EPS, succinoglycan and gluctoglycan.[4,5] It has been reported that both EPSs are required for *S. meliloti* infection of alfalfa.[4,5] Succinoglycan is a polymer consisting of several octosaccharide units (including one glactose and seven gluctose modified by one succinate and one acetate). Gluctoglycan is composed of thousand and hundreds of dimmers of one glactose and one gluctose with modification of one acetate. Their oligomers were found to work as signals on host plants. The transcriptomic data showed that *S.meliloti exoY* minus mutant (not synthesize succinoglycan) induced the elevated expression of many defense related genes on host *M. truncatula*, suggesting that succinoglycan suppresses host immunity during infection.[17] Whether the oligomer of EPS is perceived by a receptor, interacting with MAPKs to constitute a signaling pathway will be elucidated by forward genetics and biochemistry.

Most rhizobia behave type III secretion systems (T3SS) like pathogens.[18,19] Several effectors are secreted into host cells through T3SS. *Sinorhizobium* sp. NGR234 is able to infect and nodulate dozens of leguminous plants. *S.* sp. NGR234 secrets a few effectors including NopJ, NopL, NopM, NopP and NopT into host plant cells, where most of them interact with immune signaling pathways to suppress host defense responses.[21-24] For example, NopL mimics a MAP substrate to impair the MAPK signaling, while NopM is a ubiqutin ligase to reduce the ROS production induced by flg22.[20,21] Therefore, we propose that rhizobial T3SS effectors could trigger ETS to promote *Rhizobium* infection and survival in host cells.

Absolutely, phytohormones including ethylene, jasmonate and salicylic acid interplay with the signaling pathways of PTS or ETS associated with *Rhizobium* infection and survival in host legumes.[25-27] Moreover, the phytocytokine (like phytosulfokine, Clavata3-like and plant elicitor peptides) signal transduction is possibly involved in immnosuppression of *Rhizobium*-legume symbiosis.[28] In summary, immunosuppression takes place in almost all key steps of rhizobium-legume symbiosis. The signaling network could consist of PTS, ETS, phytohormones, and phytocytokines, though the molecular mechanism is not very clear now. Therefore, local immunosuppressin of plants should be considered during engineering of *Rhizobium*-nonlegume symbiosis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This work was supported by the National Key Program for Basic Research (2010CB126501, 2011CB100702) and Natural Science Foundation of China (31070218, 31370277) to L. L.

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