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Tissue-specific down-regulation of RIPK 2 in *Mycobacterium leprae*-infected nu/nu mice

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RIPK 2 is adapter molecule in the signal pathway involved in Toll-like receptors. However, there has been no reported association between receptor-interacting serine/threonine kinase 2 (RIPK 2) expression and the infectious diseases involving mycobacterial infection. This study found that its expression was down-regulated in the footpads and skin but was up-regulated in the liver of *Mycobacterium leprae*-infected nu/nu mice compared with those of the *M. leprae* non-infected nu/nu mice. It was observed that the interlukin-12p40 and interferon-γ genes involved in the susceptibility of *M. leprae* were down-regulated in the skin but were up-regulated in the liver. Overall, this suggests that regulation of RIPK 2 expression is tissue-specifically associated with *M. leprae* infection.

Key words: Interferon-γ, Interleukin-12, *Mycobacterium leprae*, Receptor-interacting serine/threonine kinase 2

The receptor-interacting serine/threonine kinase 2 (RIPK 2) is a serine/threonine kinase[1] that carries the caspase activation and recruitment domain at its carboxy terminus. It has recently been reported that RIPK 2 is essential for signalling through the Toll-like receptor family members, which are central components of the innate immune system.[2] However, the association between RIPK 2 and *Mycobacterium leprae* infection has not been studied. In order to examine the correlation between RIPK 2 and the *M. leprae* infection, RIPK 2 and cytokine mRNA expression using real-time reverse transcription-polymerase chain reaction was conducted.

Female specific pathogen free BALB/c-nu/nu mice, 3–4 weeks of age, were purchased from the Charles River Inc. (Yokohama, Japan). A dose of 1×10^6 of *M. leprae*, strain Thai 53, was inoculated into the hind footpad of the mice. The *M. leprae*-infected and non-infected nude mice were sacrificed at 18 months after the inoculation. All the procedures were carried out in accordance with the NIH Guidelines for the Care and use of Laboratory Animals. Total RNA was simultaneously separated with TRI reagent (Sigma, St. Louis, MD, USA) according to the manufacturer's protocol. The total RNA (1 μg) from the footpads, liver, and skin of the *M. leprae*-infected and non-infected nude mice was reverse-transcribed in a total volume of 20 μl using MMLV (BD Science, Palo Alto, CA, USA) with the random hexamers as the primer. Aliquots of the reverse-transcriptase products were amplified in the reaction mixture containing iQ™ SYBR® Green Supermix, 0.2 μM of each primer using iCycler iQ™ (BIO-RAD, Hercules, CA, USA) according to the manufacturer's instructions. The primers were

Table 1. RIPK 2 and inflammatory cytokine mRNA expression

Gene	Skin		Footpad		Liver	
	M. leprae non-infected nu/nu mouse	*M. leprae*-infected nu/nu mouse	*M. leprae* non-infected nu/nu mouse	*M. leprae*-infected nu/nu mouse	*M. leprae* non-infected nu/nu mouse	*M. leprae*-infected nu/nu mouse
RIPK 2	1	0.56	1	0.71	1	2.1
IL-12p40	1	0.19	0*	0*	1	15.18
IFN-γ	1	0.11	0*	0*	1	1.90

RNA was prepared from the footpads, liver, and skin of the *M. leprae*-infected and non-infected nude mice. The expression of RIPK 2, IL-12p40, and IFN-γ was determined by real-time polymerase chain reaction. The amounts of RIPK 2 and cytokine mRNA are expressed as a value relative to those in *M. leprae* non-infected nu/nu mice. The quantity of specific mRNA was measured at the point where the iCycler IQ™ system detected the threshold cycle of the exponential phase of polymerase chain reaction accumulation, which was normalized to the β-actin expression level in each tissue sample. * 'undetectable'.

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used for the RIPK2 were 5′-ATGATGTCACTCTCTC-AATCAC-3′ and 5′-CAAATGTTCTCAGAACTGGTTC-3′, and commercial primers were purchased for interleukin (IL)-12p40, interferon (IFN)-γ genes, and β-actin as a control (R&D system, Minneapolis, Minnesota, USA).

In order to determine the relative expression level of RIPK 2 and cytokines, real-time polymerase chain reaction was performed on the tissue from the *M. leprae*-infected and non-infected nude mice (Table 1). In a comparison with the skin and footpad of the *M. leprae* non-infected nude mice, the RIPK 2, IL-12p40, and IFN-γ mRNA were down-regulated by 0.56-fold and 0.19-fold, and 0.11-fold and 0.71-fold in the skin and footpad of the *M. leprae*-infected nude mice, respectively. However, the IL-12p40 and IFN-γ mRNA were not expressed in the footpads of both mice. In contrast, the RIPK 2, IL-12p40, and IFN-γ mRNA were up-regulated by 2.1-fold, 15.18-fold and 1.90-fold in the liver of the *M. leprae*-infected nude mice.

In this study, there are sharp contrasts between the IL-12p40 and IFN-γ mRNA expression levels according to RIPK 2 mRNA expression level of the liver and skin in *M. leprae*-infected nude mice. The down-regulation of RIPK 2 may result in the diminished expression of the immune response against mycobacterial infection. Correspondingly, this issue will be clarified in future studies using the primary cells to determine the involvement of the RIPK 2 signal pathway throughout Toll-like receptors to *M. leprae* infection. This study suggests that the RIPK 2 gene expression might tissue-specifically associate with *M. leprae* infection.

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