Production of Recombinant Kinesin-Related Protein of *Leishmania donovani* and its Application in the Urine-Based ELISA for the Diagnosis of Visceral Leishmaniasis

ISLAM MOHAMMAD ZAHIDUL[1] 高木 秀和[1] 伊藤 誠[1] 橋口 義久[2] 木村 英作[1]
愛知医科大学 医学部 寄生虫学教室[1] 高知大学 医学部 社会医学講座 寄生虫学教室[2]

For the serodiagnosis of visceral leishmaniasis (VL), a recombinant antigen, rK39 (a part of *Leishmania chagasi* kinesin related protein), has shown very good sensitivity and specificity. Here we report the production of recombinant kinesin-related protein with a molecular weight of 42-kDa (rKRP42) of *L. donovani*, a homologue to rK39, and the value of this antigen in urine-based ELISA for the diagnosis of VL.To obtain the gene coding the rK39 homologue, PCR amplification was performed from *L. donovani* genomic DNA. Then the amplified PCR product was subcloned in pBluescript KS(-) and sequenced. The PCR product was 1071 bp, which was just one repeat (39 amino acid) larger than the rK39 antigen. Amino acid sequence showed 89.3% identity and 98.7% homology to the rK39 antigen. The PCR product was cloned in pTYB12 expression vector. The rKRP42 was purified with Ni-NTA column, then with Chitin column, and used in the urine-based ELISA for the diagnosis of VL. The ELISA showed 94.3% sensitivity(66 positives among 70 VL samples) and 100% specificity (no positive among 80 Japanese control samples and 40 malaria samples). This assay system showed similar sensitivity and specificity to our previously reported urine-based ELISA with acetone-treated *L. donovani* promastigotes antigen. The use of this rKRP42 will ensure the stable supply of antigen.

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MOHAMMAD ZAHIDUL ISLAM
Dept of Parasitology, Aichi Medical Univ School of Medicine, Aichi, Japan