To analyze the chromosomal region around *Jsr*, the screening of the YAC library with *D5Mit22* and the BAC library with STS marker, 11MMHAP75FRD8. seq, locating in the same locus of *Jsr* was examined. The length of 380kb in BAC contig including *Jsr* was constructed; however, YAC clones were inappropriate because of deletion. It was expected that causal gene of *Jsr* was clarified by further analysis of BAC clones isolated in the present study.

Analysis of the relationship between antimicrocidal activity against *Salmonella typhimurium* and nitric oxide synthesis related with mouse natural resistant-associated macrophage protein1 gene (*Nramp1*)

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The ability of a host to resist infection with a wide range of viral, bacterial, and parasitic pathogens is strongly influenced by many genetic factors. The *Bcg/Ity/Lsh* gene on mouse chromosome 1 regulates priming/activation of macrophage for antimicrobial and tumoricidal activity. A candidate gene for *Bcg/Ity/Lsh* expressed in macrophages has been identified as the natural resistance-associated macrophage protein 1 (*Nramp1*). Macrophages are concerned with antimicrocidal activity by numerous cytokine-induced nitric oxide (NO) productions. In the present study, the relationships among *Nramp1*, antimicrocidal activity, NO production and tumor necrosis factor alpha (TNF-α) were analyzed with the experiments using peritoneal and splenic macrophages, isolated from *Nramp1* congenic and/or TNF-α knockout mice.

Peritoneal macrophage from *Nramp1* congenic, the resistant (*Nramp1*^{r}) and the susceptible (*Nramp1*^{s}) mice, infected with six strains of *S. typhimurium*, were analyzed to examine the relationship between antimicrocidal activity and NO production. Antimicrocidal activity of *Nramp1*^{r} macrophage was higher at 3hr after infection than that of *Nramp1*^{s}. Although NO production was very low in both types of macrophages within 3hr after infection, the significant levels of NO production were detected in *Nramp1*^{r} macrophage compared to that from *Nramp1*^{s} with four strains of infection.

Peritoneal macrophages from TNF-$\alpha^{-/-}$·*Nramp1*^{r} and TNF-$\alpha^{-/-}$·*Nramp1*^{s} infected with six strains of *S. typhimurium* were analyzed to examine the influence of antimicrocidal activity by TNF-α defiency. No significant effect of TNF-α was observed within 3 hrs after infection at all strain of *S. typhimurium*. At 24 hrs after infection, except for two strains of bacteria showing markedly weak antimicrocidal activity, *S. typhimurium* was proliferated remarkably in both types of TNF-$\alpha^{-/-}$ macrophages, compared to TNF-$\alpha^{+/-}$ macrophages. The production of NO was significantly low throughout the infection. In splenic macrophages, after stimulation with IFN-γ (10U/ml), LPS(10mg/ml) or with IFN-γ (10U/ml) + LPS(10mg/ml), NO production in *Nramp1*^{r} was higher than those in *Nramp1*^{s}, but not in TNF-$\alpha^{-/-}$ splenic macrophages, suggesting similar results as peritoneal macrophages.

It was suggested in both peritoneal and

splenic macrophages that *Nramp1* plays some important roles for the resistance in early stage of infection, probably changing the intra-phagosomal conditions and for the antimicrocidal activity via cytokine-NO pathway in late stage of infection. Additionally it was confirmed that TNF-α was not associated with antimicrocidal activity in early stage of infection, but it was essential for NO synthesis from macrophages.

Isolation and identification of a novel compound from garlic and its oxidative effects on canine erythrocytes

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Garlic (*Allium sativum*) has been reported to cause experimental hemolytic anemia in dogs with the appearance of an abnormal erythrocyte named eccentrocyte characterized with an asymmetric distribution of hemoglobin in the peripheral blood. In the present study, three compounds which were responsible for the garlic-induced hemolytic anemia were isolated from boiled garlics. One of them was identified by spectrum analysis of its structure and the oxidative effects of the compound on canine erythrocytes were investigated.

Three compounds (compound 1–3) possessing an oxidative effect on canine erythrocytes *in vitro* were isolated from the garlic extract by chromatography using various columns. Compound 2 was characterized as a novel sulfur-containing compound, sodium 2-propenylthiosulfate (2PTS), by means of analysis of nuclear magnetic resonance and mass spectra.

When canine erythrocytes with hereditary high reduced glutathione and potassium concentrations (HK RBCs) and normal erythrocytes (LK RBCs) were incubated with 5 mM synthetic 2PTS at 37°C for 4hrs, the oxidative damage was more severe in HK RBCs than that in LK RBCs. The oral administration of synthetic 2PTS for 7 days to clinically normal dogs resulted in a mild hemolytic anemia. The count (0.7%) of eccentrocytes in the peripheral blood of these dogs was considerably lower than that (approximately 10%) in dogs fed boiled garlic.

These results suggest that 2PTS is one of the causative agents of garlic-induced hemolytic anemia, and also suggest that 2PTS does not play an important role in garlic-induced hemolytic anemia because the hematological changes, especially the eccentrocytes count, after administration of 2PTS were different from those in dogs fed boiled garlics.