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splenic macrophages that *Nrampl* plays some important roles for the resistance in early stage of infection, probably changing the intra-phagosomal conditions and for the antimicrobial activity via cytokine-NO pathway in late stage of infection.

Additionally it was confirmed that TNF- $\alpha$  was not associated with antimicrobial 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.