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After *S. typhimurium* infection, iNOS mRNA was found by RT-PCR analysis in the *Nramp1*^r macrophages at 1-9hr but not at 24hr after infection, whereas it was detected in the *Nramp1*^s macrophages throughout 1-24hr after infection. TNF- α mRNA was observed in all macrophages except for TNF- α ^{-/-} macrophages.

These results suggested that *Nramp1* plays

an important role in NO production in the case of cytokine induction and in the antimicrobial activity in the early phase of infection, in spite of the fact that these two functions by *Nramp1* have no correlation. Additionally, it was confirmed that TNF- α was not directly related to *Nramp1* functions, but rather to NO production by macrophages.

Isolation and identification of hemolysis factors from onion juice incubated with ruminal fluid of sheep

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Onions (*Allium cepa*) are known to cause Heinz body hemolytic anemia, called "onion poisoning" in domestic animals. In ruminants, it has been suggested that microflora could produce metabolites from the onion resulting in the poisoning. In this study, several compounds suspected to be a cause of onion poisoning in sheep were isolated from onion juice incubated with ruminal fluid.

A mixture of ruminal fluid and onion juice was incubated at 38.5°C for 72 hours under anaerobic conditions. After the incubation, the fluid mixture was centrifuged and the supernatant fluid was collected. This supernatant fluid was concentrated under reduced pressure and partitioned with diethyl ether. The ether extract was dried under reduced pressure and fractionated by chromatography. Each fraction was dried and added to a sheep erythrocyte suspension. After the incubation of the cell suspension for two hours at 38.5°C, the MetHb concentration in sheep erythrocytes was measured as an indicator

of oxidative damage of the red blood cells caused by the ether extract.

Three compounds, lactic acid, phenyllactic acid and leucic acid, were identified in the extract by spectrum analysis of their structures. These compounds were D-forms and L-forms.

In the analysis of the amino acids in onion juice, threonine and glycine were found to be present in moderate quantities as free and peptides forms, respectively. Phenyllactic acid and leucic acid were also present in moderate quantities as free forms. These amino acids in the onion were thought to be substrates for these compounds produced by rumen bacteria. Threonine and glycine are able to be substrates for lactic acid, and phenylalanine and leucine were for phenyllactic acid and leucic acid production, respectively.

From these results, it was suggested that lactic acid, phenyllactic acid and leucic acid produced from onions by rumen bacteria may be the causative agents of onion poisoning.