transferred DNA-liposome complex was not expressed as protein, and to discover a way to express Fas antigen in vivo.

Analysis of the relationship between disinfection and nitric oxide synthesis in macrophages from the *Nramp1* congenic mouse

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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 numerous genetic factors. The *Bcg/Ity/Lsh* gene on mouse chromosome 1 regulates priming/activation of macrophages 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 (*Nramp1*) by positional cloning and full-length sequence analysis. Macrophages are concerned with antimicrocidal activity through cytokine-induced nitric oxide (NO) production. In the present study, the relationships among *Nramp1*, antimicrocidal activity, NO production and TNF-α were analyzed in experiments using isolated peritoneal macrophages.

Nramp1 congenic resistant (*Nramp1*^{r}) and susceptible (*Nramp1*^{s}) mice were created by mating C57BL/6 (a susceptible strain) to C3H/He (a resistant strain), since preliminary experiments revealed that genetic background influenced the real functions of the *Nramp1* gene. Additionally, for analysis of the relationship between *Nramp1* and TNF-α, TNF-$\alpha^{-/-}$·*Nramp1*^{r} and TNF-$\alpha^{-/-}$·*Nramp1*^{s} mice were prepared by mating TNF-α knockout and *Nramp1*^{r} mice. It was noted that *Nramp1*^{r} macrophages possessed higher potentiality than *Nramp1*^{s} macrophages for production of NO in the cases of IFN-γ (10U/ml) and LPS (10ng/ml) +IFN-γ (10U/ml) stimulation. The production of NO was abolished in all conditions in both TNF-$\alpha^{-/-}$·*Nramp1*^{r} and TNF-$\alpha^{-/-}$·*Nramp1*^{s} macrophages.

Macrophages from *Nramp1*^{r} and *Nramp1*^{s} infected with *Salmonella typhimurium* were analyzed to examine the the relationship between antimicrocidal activity and NO production. Antimicrocidal activity of *Nramp1*^{r} macrophages was higher at 3hr after infection than that of *Nramp1*^{s}, but no significant difference was observed at 24hr after infection. Although NO production was very small at 3hr after infection in both macrophage types, it was significantly increased at 24hr after infection in *Nramp1*^{s} macrophages compared to *Nramp1*^{r} macrophages.

Macrophages from TNF-$\alpha^{-/-}$·*Nramp1*^{r} and TNF-$\alpha^{-/-}$·*Nramp1*^{s} infected with *S. typhimurium* were analyzed to examine the influence of TNF-α deficiency on antimicrocidal activity. Antimicrocidal activity in both TNF-$\alpha^{-/-}$macrophages was at the same level as in TNF-$\alpha^{+/+}$ macrophages at 1hr after infection; however, it was noted that the extent of *S. typhimurium* infection increased in both TNF-$\alpha^{-/-}$macrophages after 3hr. The production of NO was very slight throughout the infection, and iNOS mRNA could not be detected at 24hr after infection.

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-$\alpha^{-/-}$ macrophages.

These results suggested that *Nramp1* plays an important role in NO production in the case of cytokine induction and in the antimicrocidal 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, phenylalctic acid and leucic acid produced from onions by rumen bacteria may be the causative agents of onion poisoning.