ence were found.

These results suggested that there were at least two time-specific checkpoints in spermatogenesis of the sterile mice caused by *Hst3*, resulting in apoptotic cell death. Furthermore, it was noted that cDNA clone F77 was detected as a novel gene in the present study. It was expressed in a relatively late stage of spermiogenesis (mainly in elongated spermatids), and appeared to be involved in specific spermiogenetic cell differentiation.

Transfer of Fas antigen cDNA constructed with an expression vector in cationic liposome into MRL-*lpr* mice

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Gene delivery systems using recombinant viral vectors or nonviral vectors such as liposomes have been reported. Vectors from retroviruses are known to transfer genes effectively into cells. However, the therapeutic use of viruses as gene delivery vehicles entails some problems, because retroviruses might disrupt DNA of the host cells they infect, and could cause potentially harmful results. On the other hand, nonviral methods of gene transfer would be suitable for repeated use, since they do not result in immune responses. The liposome, which is a small fatty sphere, is a nonviral vector and has been studied as well as retrovirus vectors.

It is considered that gene therapy using Fas antigen in vivo is an important way to eliminate tumours or cause them to regress through apoptosis. Mouse lymphoproliferation gene (*lpr*), which is a nonfunctional mutation of Fas antigen, has been assigned to chromosome 19. Few transcripts of Fas antigen in MRL-*lpr* mice are expressed in the thymus and liver compared to normal mice because an insertion by an early transposable element occurs in intron 2. The clinical symptoms of MRL-*lpr* mice are lymphoadenopathy, splenomegaly, hypergammaglobulinaemia and arthritis, which resemble human systemic lupus erythematosus. In this study the author designed expression vectors carrying Fas antigen cDNA in the correct orientation, named pEF-Fas and pCMV-Fas. First, it became apparent in vitro experiment and through Western blot analysis that Fas antigen protein is expressed in simian COS-1 cells transfected with lipofectin and pEF-Fas or pCMV-Fas complexes. Then the author used MRL-*lpr* mice to investigate the effect of Fas antigen expression by means of transfection of the expression vectors enclosed in cationic liposomes. After injection through the tail vein, the vectors carrying Fas antigen cDNA-liposome complex were successfully transferred mainly in the lung, liver, spleen, kidney and pancreas as detected by Southern blot analysis. Furthermore, RNA transcripts were found in lung and liver by Northern blot analysis, but were not observed in any other organ. Fas antigen protein, however, was not observed even in lung and liver by Western blot analysis. Finally the author attempted 66% partial hepatectomy to find Fas antigen protein effectively, but could not detect it. Further study is necessary to investigate the reason why the

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.