



HOKKAIDO UNIVERSITY

Title	Differential effect of the combination of Kit ^W or Kit [^] mutant Allele and the Kit ^S allele derived from <i>Mus spretus</i> on male hybrid sterility
Author(s)	Samir Ahmed Mohamed EL-Shazly
Citation	Japanese Journal of Veterinary Research, 50(1), 38-39
Issue Date	2002-05-31
Doc URL	https://hdl.handle.net/2115/2941
Type	departmental bulletin paper
File Information	KJ00002400425.pdf



macrophages was generally higher than that of *Nramp 1^s* congenic macrophages after stimulation by IFN- γ , LPS or TNF- α alone or in combination. *Nramp 1* mRNA expression in both *Nramp 1* congenic macrophages was constitutive notwithstanding cytokine stimulation. During infection with *S. typhimurium* strain 6203, *Nramp 1^r* macrophages produced a lower amount of NO due to an initial strong reaction and unsustained iNOS gene expression as compared with *Nramp 1^s* macrophages. An inhibitory effect of the *Nramp 1^r* gene on bacterial replication was also observed during the early stage of *S. typhimurium* infection, while the effect of TNF- α occurred later. NO production and iNOS expression in TNF- $\alpha^{-/-}$ macrophages were not detected from the start of the bacterial infection or at 24 h post-infection. It was also observed that *S. typhimurium* strain 6203 grew more vigorously without TNF- α , especially in *Nramp 1^s* macrophages. These data therefore demonstrate that there is cooperation of the *Nramp 1* and *Tnfa* genes in NO production and an inhibitory effect in response to *S. typhimurium* infection.

In the second part of the study, after IFN- γ and LPS stimulation, the strains NZB/N, DBA/2N, AKR/N and A/J showed signifi-

cantly lower NO production, NJL, 129/J, MOG, SJL/J, CBA/N and NOD/Shi had moderate amounts, and C3H/He and SPR had higher levels as compared to the other mice. The F1 progeny of A/J \times C3H/He and AKR/N \times C3H/He showed significantly higher NO production, whereas the F1 progeny of DBA/2N \times C3H/He produced a relatively low amount. Furthermore, the backcross progeny from F1 showed variations in NO production, and it was therefore speculated that the regulation of NO production is polygenic. Genetic typing experiments related to NO production in the backcross progeny demonstrated significant deviations to some genetic microsatellite markers. Sequencing of the iNOS promoter regions of the *Nramp 1^r* strains to examine the relationship with NO production revealed that MOG and SPR strains had substitutions within the NF- κ B and the γ -IRE, respectively.

These experiments therefore suggest that there are several factors involved in NO production and its function against infection. The use of more comprehensive experiments is suggested to further elucidate the main factors responsible for NO production and its activity against other infectious organisms.

The original paper of this thesis appeared in *J. Interf. Cytok. Res.*, 21 : 53-62 (2001) and *Biochem. Gen.*, 39 : 379-394 (2001).

Differential effect of the combination of *Kit^w* or *Kit^{w-v}* mutant Allele and the *Kit^s* allele derived from *Mus spretus* on male hybrid sterility

Samir Ahmed Mohamed El-Shazly

Laboratory of Experimental Animal Science, Department of Animal Disease Control,
Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan

Sterility in interspecific hybrids usually affects the heterogametic sex. This is known

as Haldane's rule which applies to male mammals. In mice, six hybrid sterility (*Hst*) loci have been defined. However, the *w*-series of *Kit* alleles is based on mutations of the proto-oncogene *Kit* that encodes a transmembrane tyrosine kinase receptor for the mast cell growth factor. The *Kit* gene is located at position 42 on chromosome 5 and plays a wide range of important roles in pigmentation, mast cell growth, hematopoiesis and gametogenesis. In this study, we investigated the effect of the combination of some mutant *Kit* alleles and the *Kit^S* allele derived from Spanish wild mice (*Mus spretus*).

In the first part of the study, two congenic strains, C57BL-*Kit^W* and C57BL-*Kit^S*, were generated. The *Kit^W* allele originated from strain WB-*Kit^W* and the *Kit^S* allele from *Mus spretus*. The *Kit^W/Kit^S* males showed hybrid sterility with small testes, but the females were fertile. Development of the seminiferous tubules of the *Kit^W/Kit^S* males stopped before the spermatocyte stage and they were almost free of sperm. Mapping chromosome 5 in the C57BL-*Kit^S* congenic strain showed that the region between positions 42 and 44 was derived from SPR. Eleven amino acid substitutions of the *Kit^S* cDNA were detected by comparison with the sequence data of the +*Kit* cDNA from C57BL; seven were in the extracellular domain, one in the transmembrane domain, two in the kinase I domain, and one in the carboxy-terminal tail. The *Kit* mRNA derived from both *Kit^W* and *Kit^S* alleles was expressed in the sterile testes of the *Kit^W/Kit^S*

males.

In the second part of the study, reproduction of the combination of *Kit^{W-v}* and *Kit^S* alleles was examined. The *Kit^{W-v}/Kit^S* male was fertile and the histological structure was normal; the seminiferous tubules all showed normal developmental phases of spermatogenesis. The postnatal development of the testis at 8, 12, 16, and 20 days was also studied in the fertile +*Kit*/+*Kit* and *Kit^{W-v}/Kit^S* males and the sterile *Kit^W/Kit^S*. The results showed that at 8 days there was no noticeable difference among the three genotype combinations, while from 12 to 20 days the spermatogenesis in the *Kit^W/Kit^S* male nearly stopped before the meiosis stage. The expression of the *Kit* receptor protein from the *Kit^S* allele in the sterile testis of the *Kit^W/Kit^S* male was confirmed using Western blot analysis. The *Kit* ligand derived from *Mus spretus* showed two amino acid changes in the extracellular domain compared to that from C57BL.

In conclusion, it is possible that in addition to a low level of surface expression of *Kit* protein in *Kit^W/Kit^S*, the presence of seven amino acid changes in the extracellular domain from *Kit^S* allele reduces the binding affinity to the ligand from C57BL, decreases the following signal transduction, and causes male sterility. On the other hand, in congenic C57BL-*Kit^S/Kit^S* and C57BL-*Kit^{W-v}/Kit^S* males, the 100% expression of surface protein could compensate for the reduction of the affinity to the ligand, as they are fertile.

The original paper of this thesis appeared in *Bioch. Genet.*, 39 : 127-137 (2001) and *Develop. Growth Differ.*, 43 : 611-617 (2001).