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Development of *in vivo* Gene expression method using recombinant Baculovirus in mouse testis

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In order to attain gene expression in vivo in the testis of living mice, we applied *Autographa californica* multinuclear polyhedrosis virus (baculovirus). By infection with a baculovirus containing a green fluorescent protein (GFP) cassette to GC-1, Cos-7 and Hela cells, we found that these cell types can be infected efficiently. Although it is accordant with previous report that Cos-7 and Hela are well characterized for infection of recombinant baculovirus, infection of recombinant baculovirus to GC-1 spermatogonia cells are considerable in the aspect of production of transgenic animal using transgenic spermatogonia transplantation. Intra-tunica albuginia injection of GFP-baculovirus of living mouse testis also show a high level GFP expression in somatic cells including leydig cells. Injection of GFP-baculovirus into seminiferous tubule through rete testis do not show any green germ cells or spermatozoa. However, RT-PCR analysis of this testis clearly show that GFP gene is expressed in seminiferous tubule and caput epididymis at 7 days post injection. Furthermore, we have produced several offspring by natural mating between GFP-baculovirus injected male and normal female. The male mice began the mating after 3 days of post injection, and 7 out of 13 offsprings had GFP gene by tail genomic DNA analysis, whereas vector or PBS injected mouse did not. We postulate that it could be due to insufficient time for infected germ cells or spermatogonia cells developing to spermatozoa. Therefore, we suggest that injected recombinant baculovirus may enter or attach to

spermatozoa in testis or epididymis, and join the normal fertilization process, and transmit viral coding gene. In conclusion, we have developed the *in vivo* gene expression system in living mouse testis using recombinant baculovirus, that could be applied to gene therapy in testis or production of male derived transgenic animal.

Keywords: ***recombinant baculovirus, intra-tunica albuginea virus injection, GC-1 cell, gene therapy***