Croatian Medical Journal

CMJ - March 1996 (Volume 37, Number 4)

Germline Transmission in IL-9 Knockout Mice: a Practical Approach to Intrastrain and Interstrain Variability

Anto Bagiæ
Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, MD, USA

Aim. Analysis and comparison of the contribution of 129 and C57BL/6 (BL/6-III) embryonic stem (ES) cell clones in the coat and germline of chimeras, and development of a strategy to optimize germline transmission.
Methods. Interleukin-9 gene was inactivated in 129 and C57BL/6 (BL/6-III) ES cells using a gene-targeting technique, and two confirmed clones from each cell background were injected into C57BL/6 and BALB/cByJ blastocysts, respectively. After reexpansion, the injected 3.5 days post coitum (dpc) embryos were orthotopically transferred into the uterus of 2.5 dpc pseudopregnant foster mothers. The pups were born seventeen days later. At three weeks of age, they were weaned, ear-tagged, and the level of coat chimerism was visually assessed. At 7 weeks, male chimeras were test-mated for germline transmission with females of the host-embryo background, and the transmission was confirmed on the basis of coat color of the F1 offspring.
Results. Two 129 clones, 1F6 and 3G1, containing IL-9 gene mutated by homologous recombination, were identified. 106 (1F6) and 112 (3G1) C57BL/6 blastocysts were injected and 16 (12 males) and 17 (13 males) chimeras identified, respectively. Ten male chimeras from 1F6 clone transmitted the IL-9 mutation in the germline and produced brown (strain 129 origin) pups after mating with C57BL/6 (black) females. In contrast, none of the 13 male chimeras from 3G1 clone were germline chimeras. From 104 (3G9) and 107 (3E11) BALB/cByJ blastocysts injected with BL/6-III clones, we obtained 7 (2 males) and 3 (all males) chimeras respectively, and of these, none transferred the IL-9 mutation in the germline.
Conclusions. Our data confirm the importance of proper testing of the parental cell line and all derived clones with respect to germline transmission. Intrastrain and interstrain variability remains the main obstacle to germline chimera production. However, new approaches to this problem may significantly increase the level of success.

Key words: animals, transgenic; embryonic induction; gene targeting; IL-9; recombination, genetic; stem cells