TRANSGENIC MICE IN IMMUNOBIOLOGY

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Contents

Summary

Strategies to generate transgenic mice with defined genetic alterations have evolved rapidly. Highly sophisticated strategies have been developed in which the activity of a selected gene can be spatially and temporally controlled. A large amount of information has been gained by utilizing transgenic mice in the study of complex biological processes such as the immune system. This article describes advances in the technologies available to manipulate the mouse genome. Moreover, applications of these technologies in the investigation of basic immunology and in modeling immunological disorders are outlined.

1. Introduction

There has been a revolution in the development of strategies used in the manipulation of the mouse genome. These technologies represent fascinating tools with which to investigate biological processes in mammals (see *Mammalian Cell Culture* and *Genetic Engineering of Mammalian Cells*). Although the ability of the mouse to model human diseases has been criticized, a consensus exists that the majority of physiological and chromosomal events are mediated in a similar manner in both species. Since both

species also share similarities in their anatomy, this can be utilized in the examination of developmental processes in humans. Relevant *in vivo* models are essential in developing strategies to prevent or treat human diseases as *in vitro* systems can never provide the complex and tightly regulated environment of organs and cells present in the living organism. Furthermore, sophisticated biological processes such as the immune system cannot be investigated in lower organisms (e.g., bacteria, nematode worms, or fruit fly), they must be analyzed in organisms that possess such processes.

Several human immunological diseases are caused by chromosomal aberrations such as gene deletions, duplications, inversions, translocations, and point mutations (see *Methods in Gene Engineering*). These events can impair the function of the gene product directly, or they can interfere with gene regulation. Chromosomal defects can be spontaneously induced, but exposure to external factors, e.g., X-ray radiation and certain chemicals can substantially increase the frequency of gene aberrations. Gene-manipulated mice with defined alterations in their genome can be exploited in modeling gene-mediated human diseases. Such mice are also useful in the investigation of which biological processes are required to respond efficiently against pathogens, or to examine homeostasis in the organism. This review describes advances in the transgenic technologies available to manipulate the mouse genome. Moreover, applications of these techniques in the investigation of basic immunology and immunological diseases are outlined.

2. Strategies to Generate Transgenic Mice

Several mouse strains with spontaneous mutations have been identified over the years and permanent colonies of these mutant strains have been produced, but because of the stochastic nature and the low frequency of the spontaneous mutation this approach is not suitable for the generation of specific gene defects. Exposure to certain chemicals and radiation has also been used to increase the mutation frequency in mice, but the unpredictable nature of the mutation process makes this approach difficult to use in an efficient way. In the early 1980s, investigators introduced cloned DNA directly into fertilized mouse eggs by microinjection and were able to generate transgenic mice with stable integration of foreign DNA into the host genome. The limitation of this approach was that transgene integration was a random process (see *Transgenic Animals*). The revolution in gene targeting occurred in the late 1980s by an ingenious combination of two different techniques: 1) the generation of the pluripotent embyryonic stem (ES) cells in culture and 2) the development of *in vitro* gene targeting methodology in mammalian cells by homologous recombination. Thereafter tremendous progress was made in the generation of gene-manipulated mice. In early 1991, there were seven targeted mutations described in the literature whereas a mere six years later more than 700 mutations had been reported. Strategies to manipulate the mouse genome have evolved rapidly, utilizing more and more sophisticated techniques that can be applied in the study of complex biological processes.

2.1. Gene targeting with homologous recombination

The defining property of ES cells is their stable maintenance of a pluripotent differentiation potential, as demonstrated by the expression cell markers for the

pluripotent cell but not those of differentiated cells. ES cells can be cultured in large numbers which permits the genetic modifications of these cells in culture. ES cells also have a remarkable ability to colonize the host embryo, which is crucial in transferring genetic information from ES cells into the germline. Advances in controlling ES cell differentiation have also provided insights into cell biology and the determination of cell fate.

In homologous recombination, the enzymatic machinery of the recipient cells replace homologous endogenous DNA sequences with exogenous DNA sequences. By utilizing specific targeting vectors that are transferred into ES cells, the desired endogenous gene can be permanently eliminated, resulting in gene knockout cells (Figure 1A). Alternatively, targeting vectors can be designed to transfer foreign DNA into the desired site in the host DNA without disrupting the endogenous gene resulting in knocking cells (Figure 1B).

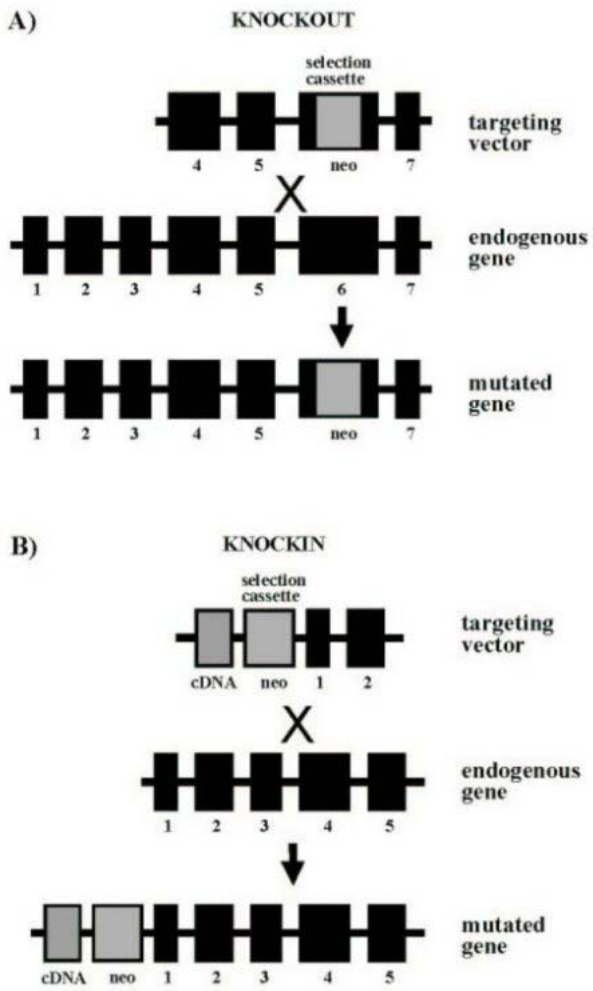

Figure 1. Generation of knockout and knockin cells by homologous recombination. A) Targeting vector contains sequences homologous to endogenous gene and a selection cassette (neo) inserted into an exon. Homologous recombination event replaces genomic sequences by vector sequences and disrupts endogenous exon by neo. B) Knockin strategy. DNA of interest will be inserted in-frame in an endogenous exon to be

targeted. The transgene will be expressed in the desired place of the host DNA without disrupting the endogenous gene.

Because the frequency of the homologous recombination event is low, investigators have devised strategies to enrich cells in which homologous targeting events have taken place. The most frequently used approach is a "positive-negative" selection strategy (Figure 2). This procedure has two components: a) a positive selectable gene to enrich recipient cells that have incorporated the targeting vector somewhere in their genomes, and b) a negative selectable gene to eliminate cells in which the targeting vector has been incorporated randomly into nonhomologous sites in the genome. To achieve this kind of selection, the transfected ES cells are cultured in a medium containing a drug for positive selection which kills cells that lack the protective drug resistance gene, derived from the targeting vector, and a drug for a negative selection destroying cells containing a negative selectable gene that is incorporated into genomes only if the targeting vector is incorporated at nonhomologous sites. This strategy guarantees an enrichment of targeted versus nontargeted ES cells by up to 50-fold and is the approach used to generate most of the gene-knockout mice reported so far.

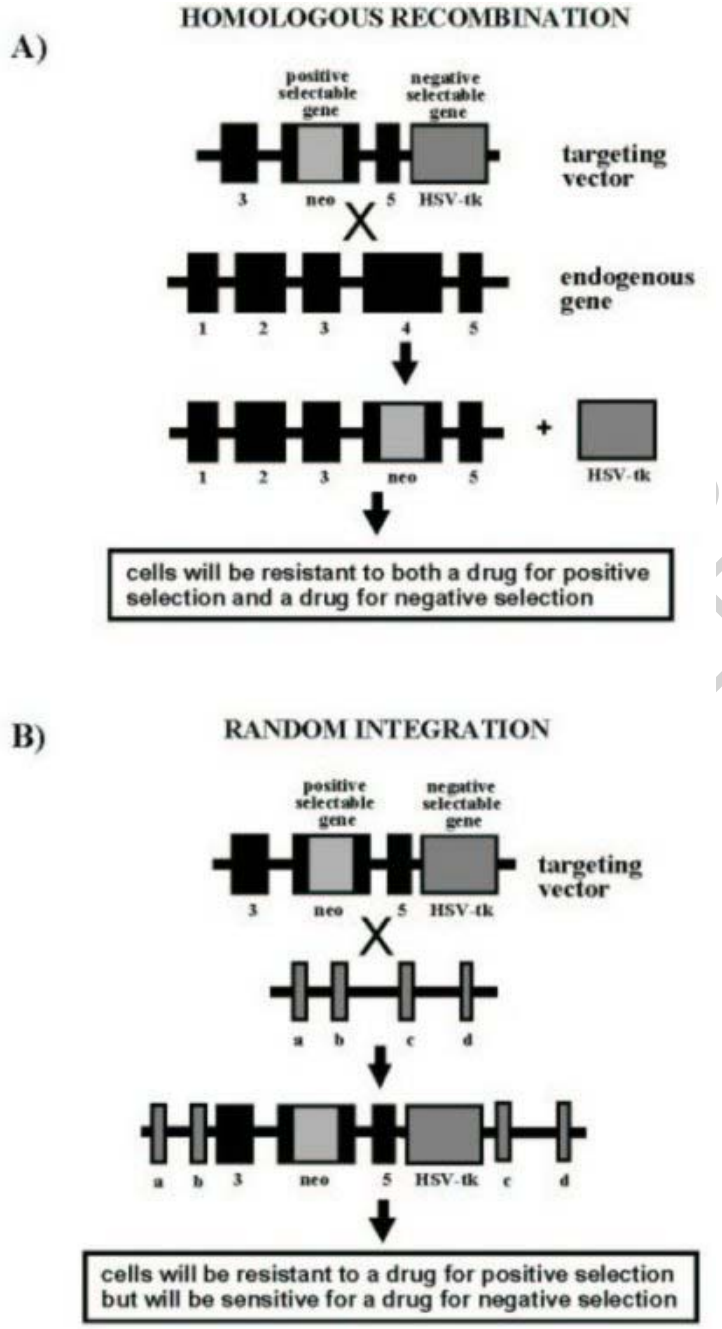

Figure 2. Positive-negative selection strategy for enrichment of targeted ES cells. A) In a homologous recombination event, only a positive selectable gene (neo), derived from the targeting vector, is incorporated into the desired place in the genome, whereas a negative selectable gene (HSV-) is not incorporated. This generates targeted cells that are resistant both to a drug for positive selection and a drug for negative selection. B) In a random integration event both a positive selectable gene (neo) and a negative selectable gene (HSV-) are incorporated into the genome. This generates cells that are resistant to a drug for positive selection but are sensitive to a drug for negative selection.

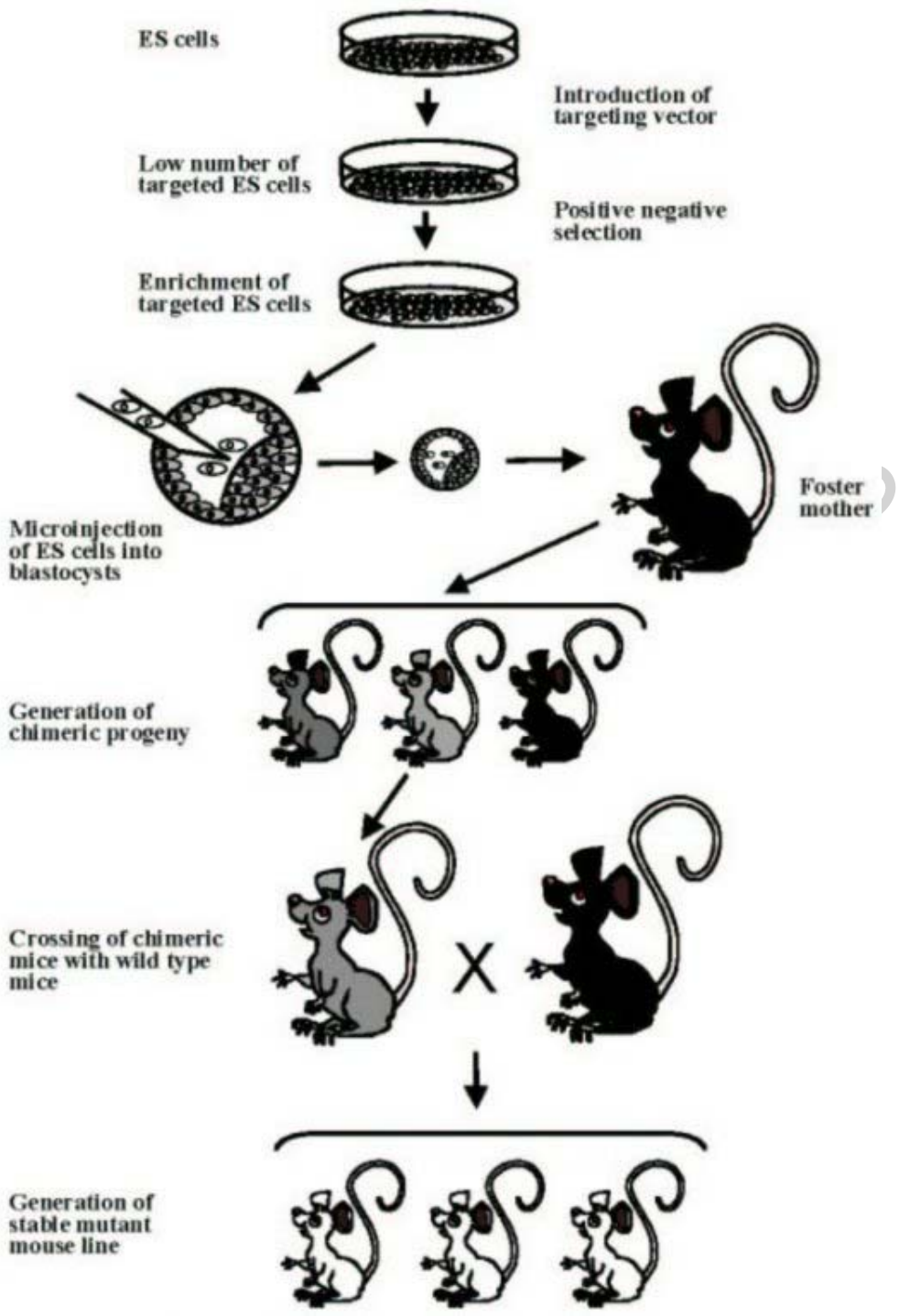

Figure 3. Generation of gene targeted mice. The first step involves introduction of targeting vector into ES cells. A positive-negative selection strategy is used to enrich the targeted ES cells. Selected targeted ES cells are injected into blastocysts which are in turn surgically transferred into the uterus of foster mothers generating chimeric progeny. Chimeric mice are crossed with wild type mice to establish a stable germline transmission of transgene. The progeny derived from the chimeras and wild type mice are characterized and a stable mutant mouse line carrying the transgene is generated.

The next step in the generation of gene-targeted mice includes *in vitro* microinjection of selected ES cells which contain the desired gene modification into mouse preimplantation embryos, termed as blastocysts (Figure 3). Blastocysts are in turn surgically transferred into the uterus of hormone-treated foster mothers, resulting in generation of chimeric progeny (Figure 3). Chimeric mice have mosaic-like tissues composed of a random distribution of wild type cells and knockout cells. To facilitate isolation of the chimeric progeny with a high degree of gene-targeted cells, the ES cells and the recipient blastocysts are derived from mice with different coat color alleles. This allows evaluation of the extent of chimerism by the coat color chimerism. Chimeric mice are then crossed with wild type mice to establish a stable germline transmission of the modified genome (Figure 3). The progeny derived from the chimeras and wild type mice are characterized and the mice with gene modification are selected. These mice are used to generate the stable mutant mouse line that carries the desired gene modification in their germline.

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Bibliography

Adorini L., Gregori S. and Harrison L.C. (2002). Understanding Autoimmune Diabetes: Insights from Mouse Models. *Trends Mol Med* **8**, 31–38. [This article describes the use of mouse models in the studies of autoimmune diabetes.]

Baron U., Freundlieb S., Gossen M. and Bujard H. (1995). Co-regulation of Two Gene Activities by Tetracycline Via a Bidirectional Promoter. *Nucleic Acids Res* **23**, 3605–3606. [This article describes the use of a tetracycline-based system in controlling expression of two target transgenes.]

Capecchi M.R. (2001). Generating Mice with Targeted Mutations. *Nat. Med.* **7**, 1086–1090. [Together with the article by Smithies (2001) this paper describes the early history of generating mice with targeted mutations.]

Chen J., Lansford R., Stewart V., Young F. and Alt F.W. (1993). RAG-2-deficient Blastocyst Complementation: An Assay of Gene Function in Lymphocyte Development. *Proc. Natl. Acad. Sci. USA* **90**, 4528–4532. [This article contains the first description of the RAG complementation assay.]

Costantini F. and Lacy E. (1981). Introduction of A Rabbit Beta-Globin Gene into the Mouse Germ Line. *Nature* **294**, 92–94. [Generation of the first transgenic mice by microinjection is described in this article.]

DiSanto J.P., Muller W., Guy-Grand D., Fischer A., Rajewsky K. (1995). Lymphoid Development in Mice With A Targeted Deletion of the Interleukin 2 Receptor Gamma Chain. *Proc. Natl. Acad. Sci. USA* **92**, 377–381. [This article describes the generation of the interleukin 2 receptor deficient mice.]

Doetschman T., Gregg R.G., Maeda N., Hooper M.L., Melton D.W., Thompson S. and Smithies O. (1987). Targeted Correction of a Mutant HPRT Gene in Mouse Embryonic Stem Cells. *Nature* **330**, 576–578. [First targeting of a gene in ES cells is presented in this article.]

Evans M.J. and Kaufman M.H. (1981). Establishment in Culture of Pluripotential Cells from Mouse Embryos. *Nature* **292**, 154–156. [This paper describes the production of pluripotent mouse embyryonic stem cells in culture conditions.]

Finotto S., Neurath M.F., Glickman J.N., Qin S., Lehr H.A., Green F.H., Ackerman K., Haley K., Galle P.R., Szabo S.J., Drazen J.M., De Sanctis G.T. and Glimcher L.H. (2002). Development of Spontaneous Airway Changes Consistent with Human Asthma in Mice Lacking T-bet. *Science* **295**, 336–338. [Generation of T-bet deficient mice is described in this article.]

Gossen M. and Bujard H. (1992). Tight Control of Gene Expression in Mammalian Cells by Tetracycline-Responsive Promoters. *Proc. Natl. Acad. Sci. USA* **89**, 5547–5551. [This paper describes the development of the tetracycline-dependent gene expression regulatory system.]

Gossen M., Freundlieb S., Bender G., Muller G., Hillen W. and Bujard H. (1995). Transcriptional Activation by Tetracyclines in Mammalian Cells. *Science* **268**, 1766–1769. [This paper describes the modified version of the tetracycline-dependent gene expression regulatory system.]

Gu H., Marth J.D., Orban P.C., Mossmann H. and Rajewsky K. (1994). Deletion of a DNA Polymerase Beta Gene Segment in T Cells Using Cell Type-Specific Gene Targeting. *Science* **265**, 103–106. [This article describes the first tissue-specific gene inactivation by the Cre/loxP system.]

Kips J.C., Tournoy K.G. and Pauwels R.A. (2000). Gene Knockout Models of Asthma. *Am. J. Respir. Crit. Care Med.* **162**, S66–70. [Review of the gene knockout mouse models used in asthma studies]

Koller B.H., Marrack P., Kappler J.W. and Smithies O. (1990). Normal Development of Mice Deficient in Beta 2M, MHC Class I Proteins, and CD8+ T cells. *Science* **248**, 1227–1230. [Together with the study by Zijlstra et al. this article describes the generation of the first Beta 2-microglobulin deficient mice.]

Kuhn R., Schwenk F., Aguet M. and Rajewsky K. (1995). Inducible Gene Targeting in Mice. *Science* **269**, 1427–1429. [Generation of the first inducible gene targeting in mice is described in this article.]

Leonard W.J. (2001). Cytokines and Immunodeficiency Diseases. *Nature Rev. Immunol.* **1**, 200–208. [Excellent review of the forms of SCID that result from defects in cytokine signaling.]

Lewandoski M. (2001). Conditional Control of Gene Expression in the Mouse. *Nat. Rev. Genet.* **2**, 743–755. [Excellent review of the conditional control of gene expression in the mouse.]

Mak T.W., Penninger J.M. and Ohashi P.S. (2001). Knockout Mice: A Paradigm Shift in Modern Immunology. *Nature Rev. Immunol.* **1**, 11–19. [Excellent review of the use of gene knockout mice in immunobiology.]

Mansour S.L., Thomas K.R. and Capecchi M.R. (1988). Disruption of the Proto-Oncogene int-2 in Mouse Embryo-Derived Stem Cells: A General Strategy for Targeting Mutations to Non-selectable Genes. *Nature* **336**, 348–352. [The generation of a general strategy to enrich for cells in which homologous gene-targeting events have occurred is described in this article.]

Mathis D., Vence L. and Benoist C. (2001). Beta-Cell Death During Progression to Diabetes. *Nature* **414**, 792–798. [Excellent review of Type I Diabetes.]

Smithies O., Gregg R.G., Boggs S.S., Koralewski M.A. and Kucherlapati R.S. (1985). Insertion of DNA Sequences into the Human Chromosomal Beta-Globin Locus by Homologous Recombination. *Nature* **317**, 230–234. [Generation of the first gene targeted mammalian cells by homologous recombination is described in this article.]

Smithies O. (2001). Forty Years with Homologous Recombination. *Nat. Med.* **7**, 1083–1086. [Together with the article by Capecchi this paper describes the early history of generating mice with targeted mutations.]

Stanford W.L., Cohn J.B. and Cordes S.P. (2001). Gene-Trap Mutagenesis: Past, Present and Beyond. *Nat. Rev. Genet.* **2**, 756–768. [Excellent review of the gene-trap mutagenesis.]

Thompson S., Clarke A.R., Pow A.M., Hooper M.L. and Melton D.W. (1989). Germ Line Transmission and Expression of a Corrected HPRT Gene Produced by Gene Targeting in Embryonic Stem Cells. *Cell* **56**, 313–321. [The first report of germ line transmission of targeted mutation by gene targeting in ES cells is presented in this article.]

Utomo A.R., Nikitin A.Y. and Lee W.H. (1999). Temporal, Spatial, and Cell Type-Specific Control of Cre-Mediated DNA Recombination in Transgenic Mice. *Nat. Biotechnol.* **17**, 1091–1096. [Description of the Cre/loxP based method which allows temporal, spatial, and cell-type-specific control DNA recombination in transgenic mice.]

Yu Y. and Bradley A. (2001). Engineering Chromosomal Rearrangements in Mice. *Nat. Rev. Genet.* **2**, 780–790. [Excellent review of the techniques used for gene-targeting in mice.]

Zheng T., Zhu Z., Wang Z., Homer R.J., Ma B., Riese R.J., Jr., Chapman H.A., Jr., Shapiro S.D. and Elias J.A. (2000). Inducible Targeting of IL-13 to the Adult Lung Causes Matrix Metalloproteinase- and Cathepsin-Dependent Emphysema. *J. Clin. Invest.* **106**, 1081–1093. [This article shows the effects of inducible overexpression of IL-13 in murine lungs.]

Zijlstra M., Bix M., Simister N.E., Loring J.M., Raulet D.H. and Jaenisch R. (1990). Beta 2-Microglobulin Deficient Mice Lack CD4-8+ Cytolytic T Cells. *Nature* **344**, 742–746. [Together with the study by Koller et al. this article describes the generation of the first Beta 2-microglobulin deficient mice.]

Biographical Sketch

Harri Alenius has an M.Sc. from the Department of Cell and Molecular Biology, University of Jyväskylä, and a Ph.D. from the Medical School of the University of Tampere, where he is also a Docent. He is the permanent Chief of the Laboratory of Immunotoxicology at the Finnish Institute of Occupational Health. Dr. Alenius was a Research Fellow at the Medical College of Wisconsin, US, and a postdoctoral fellow at Children's Hospital, Harvard Medical School, Cambridge, MA, US. He has written more than 50 articles in the field of Allergy and Immunology, and is a reviewer for *The Journal of Immunology*, *Int. Arch. of Allergy and Applied Immunology*, *J. Allergy Clin. Immunol*., *Clin. Exp. Allergy*, and *Allergy*.