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Targeted disruption of genes in mouse embryonic stem cells, incorporation of the mutation into the germline, and transmission to offspring was realized only in the late 1980s. Research in the laboratories of Mario Capecchi, Rudolf Jaenisch, Elizabeth Robertson, and Oliver Smithies pioneered this effort. It is difficult to believe, but reports of the first targeted deletion of a gene of immunological import and the generation and analysis of mice homozygous for the mutation were published only in 1990. Two papers independently reported the generation of mice in which both copies of the β_2-microglobulin (*β_2M*) gene had been disrupted (1, 2). So novel and powerful was this technology that both groups had previously published (in 1989) the derivation of the targeted embryonic stem cells, their injection into blastocysts, and the generation of mice *heterozygous* for the *β_2M* mutation (3, 4).

Disruption of the *β_2M* gene and production of "β_2M-knockout mice" confirmed in the whole animal the many previously suspected roles of the molecule. Thus, the mice lacked surface expression of the classical MHC class I H-2K and H-2D proteins because β_2M is the L chain partner for the class I H chain. Similar results had been shown previously in β_2M-deficient cell lines. The β_2M-knockout mice lacked $CD8^+$ T cells because MHC class I expression by thymic epithelial cells is required to "positively select" class I-restricted, $TCR\alpha\beta^+$, $CD8^+$ T cells. In addition, one of the papers from 1990 showed that functional expression of the neonatal Fc receptor, also known to be β_2M associated, was lacking in the knockout mice (2). Finally, both groups reported that T cells expressing a $\gamma\delta$ TCR were present in normal numbers in the spleen and thymus of β_2M-knockout mice.

Perhaps more important than these expected phenotypes, the knockout mice showed what β_2M did *not* do. The homozygous β_2M-knockout mice were healthy, and both males and females were fertile. The knockout mice had apparently normal development, ruling out previous suggestions of an involvement of β_2M or MHC molecules in a wide range of developmental and signaling functions. Whatever those functions may be, the role of β_2M was dispensable. In addition, the normal breeding behavior of homozygous β_2M-knockout mice lent no support to the suggestion that MHC class I was somehow involved in mating preferences of mice.

More important still are the later discoveries that depended on or benefited from the use of β_2M-knockout mice. The idea that NK cells would respond to "missing self" to become activated to remove MHC-deficient cells had been proposed (5). The new knockout mice provided a clear demonstration of this with the finding that it was almost impossible to reconstitute a lethally irradiated wild-type recipient with bone marrow stem cells from a β_2M-deficient donor (6). The knockout marrow was rapidly rejected. This was a novel incarnation of the MHC-linked barrier to transplantation known as the hybrid histocompatibility (Hh) phenomenon (7). Rejection of knockout marrow was shown to be due to the inability of β_2M-deficient stem cells to deliver "OFF" signals to recipient NK cells via their MHC-specific inhibitory receptors.

The β_2M-knockout mice lacked NKT cells (8, 9). These TCR-positive cells develop in the thymus and express either the CD4 coreceptor or no coreceptor. A nonclassical, β_2M-associated class I molecule, CD1, was later shown to be the ligand for this lineage (10). For NKT cells, their selection in the thymus came with a novel twist: in this case, the selecting MHC class I was expressed on double positive thymocytes themselves and not epithelial cells, as shown by the use of radiation chimeras with wild-type and β_2M-knockout partners.

The β_2M-knockout mice also contributed to our understanding of the self-peptide dependence for positive selection of $CD8^+$ T cells in the thymus. Fetal thymus lobes from mice lacking β_2M (which do not develop mature $CD8^+$ T cells) were used in an organ culture system to demonstrate that, with a polyclonal TCR repertoire, the addition of a diversity of class I-binding peptides plus exogenous β_2M was required to positively select a significant population of mature $CD8^+$ T cells (11). With stem cells bearing a single MHC class I-restricted TCR, a single peptide, related to the agonist peptide but with extremely low affinity for the TCR, was shown to be required for positive selection without inducing negative selection (12).

In 1994, β_2M-knockout mice were found to suffer from iron overload (13). Livers from the knockout mice had many times higher levels of iron than did heterozygous control animals. This mimicked the HLA-linked human condition known as hereditary hemochromatosis. Mutation in a novel class I-like gene within the HLA complex, called HLA-H, was later reported to be highly associated with the disease (14).

The β_2M-knockout mice have contributed greatly to the analysis of T cell, NKT cell, and NK cell function. Their generation in 1990 represents a landmark in biology as a whole. The knockouts ushered in the modern era of targeting genes of interest for deletion in the germline or for conditional deletion via the use of "floxed" alleles and tissue-specific or timed expression of the cre recombinase.

Disclosures

The author has no financial conflicts of interest.

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