Società Italiana di Fisiologia

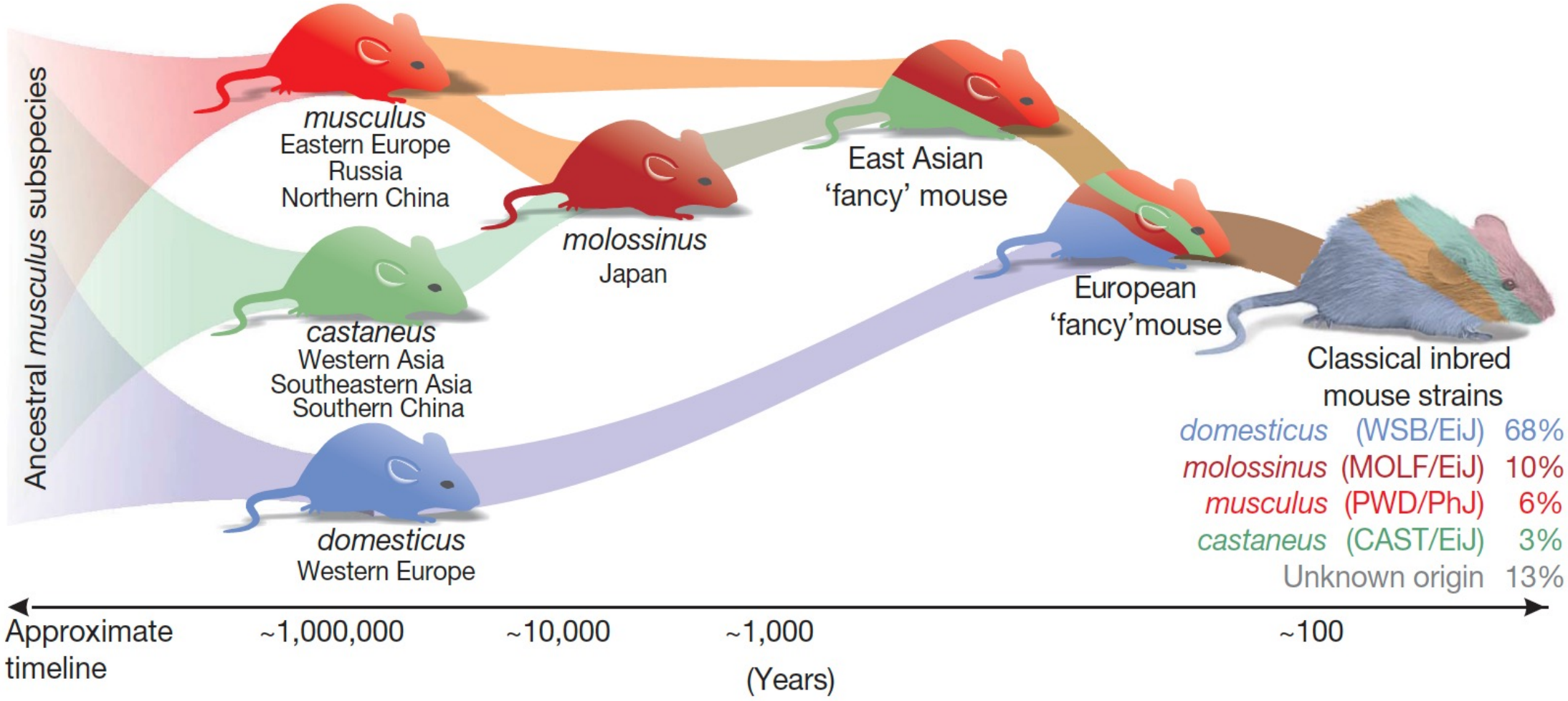
The Italian Society of Physiology

SPERIMENTAZIONE ANIMALE

MICE
Immunology studies

Thank to Federica Cavallo, University of Turin

Mice in immunology studies



Frazer KA et al., Nature volume 448:1050-1053

Mice in immunology studies: history

- During the 1700s, collecting and breeding *'fancy' mice with different coloured coats* became a popular hobby in Japan. The trend then began to find its way to Europe during the 1800s.
- In 1850, Gregor Mendel started his investigations into inheritance by breeding different colored mice.
- In 1902, Lucien Cuénot was the first to demonstrate Mendel's theories of inheritance by highlighting the genetics of coat color characteristics in mice.
- In Harvard, William Castle began his research in the same year, buying mice from a local mouse enthusiast. Together with his student Clarence Little, he produced a series of important papers on the genetics of coat color in mice.
- Clarence Little developed the inbred 'lab' mouse, which he produced by mating generation after generation of mouse siblings ➠ stabilize the genome and fix certain traits (MHC!!) through different generations.
- Over time, the mouse has become the preferred organism for research into mammalian genetics because of its rapid generation time, small size and the ease with which it can be bred.
- Now, there are over 100 different inbred strains of mice, each with a different genetic background.

Mice in immunology studies: why

- Mouse genome & human genome: about 3.1 billion base pairs. Only about 5% of the sequence consist of protein-coding regions (genes). On average, the protein-coding regions of the mouse and human genomes are 85% identical; some genes are 99% identical while others are only 60% identical. These regions are evolutionarily conserved because they are required for function. In contrast, the non-coding regions are much less similar (only 50% or less).

- The ENCODE project: launched by NIH to build a comprehensive catalog of functional elements in the human and mouse genomes. ENCODE scientists applied several genomic approaches to 123 different mouse cell types and tissues, and then compared them with the human genome.

- Gene regulation and other systems important to mammalian biology have many similarities between mice and humans. Specific DNA sequence differences linked to diseases in humans often have counterparts in the mouse genome. Genes whose expression patterns are related in one species also tend to be similarly related in the other species.

- It is relatively easy to manipulate the mouse genome, for example, adding or removing a gene to better understand its role in the body. This provides a powerful tool for modelling specific diseases when a mutated gene is known to play a role in the disease.

Inbread strains

- Obtained by inbreeding (brother by sister or parent by offspring mating) for more than 20 consecutive generations
- 98.6% of genetic loci are homozygous
- In a given inbred strain of mice, every mouse is genetically identical to every other mouse in that strain
- Animals from the same inbred strain are called syngeneic.
- In spite of the remaining genetic differences, can be regarded as genetically identical, i.e. isogeneic.
- In syngeneic strains, any member of the strain accepts tissue grafts tranplanted from any other member of the same sex of the strain without any sign of rejection, because the histocompatibility antigens of all individuals of the strain are identical.
- Due to a weak histocompatibility antigen encoded by a gene locus on the Y chromosome, grafts from male donors can be rejected in female recipients.

Mice in immunology studies

MHC locus

The MHC locus is called “H-2” in mouse.

Different inbred strains of mice differ at their MHC locus (different allelic form of the locus).

The entire locus containing all the MHC genes is usually inherited as a group.

Nevertheless, recombination within the MHC locus can occur when two different strains are crossed.

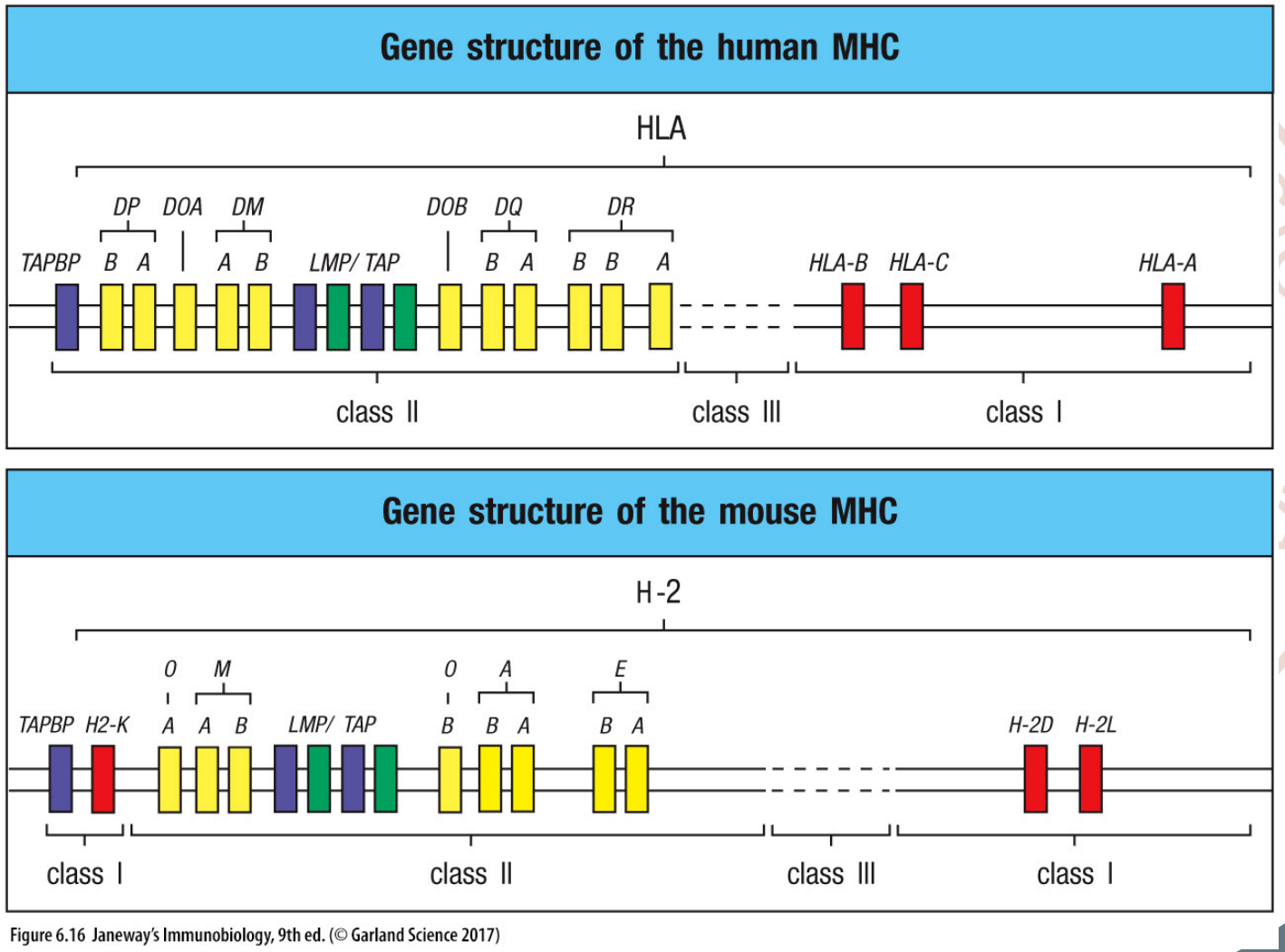


Figure 6.16 Janeway’s Immunobiology, 9th ed. (© Garland Science 2017)

Mice in immunology studies

H-2 haplotypes

TABLE 8-1 H-2 haplotypes of some mouse strains

Prototype strain	Other strains with the same haplotype	Haplotype	H-2 ALLELES K	IA	IE	S	D
CBA	AKR, C3H, B10.BR, C57BR	*k*	*k*	*k*	*k*	*k*	*k*
DBA/2	BALB/c, NZB, SEA, YBR	*d*	*d*	*d*	*d*	*d*	*d*
C57BL/10 (B10)	C57BL/6, C57L, C3H.SW, LP, 129	*b*	*b*	*b*	*b*	*b*	*b*
A	A/He, A/Sn, A/Wy, B10.A	*a*	*k*	*k*	*k*	*d*	*d*
B10.A (2R)*		*b2*	*k*	*k*	*k*	*d*	*b*
B10.A (3R)		*i3*	*b*	*b*	*k*	*d*	*d*
B10A. (4R)		*b4*	*k*	*k*	*b*	*b*	*b*
A.SW	B10.S, SJL	*s*	*s*	*s*	*s*	*s*	*s*
A.TL		*t1*	*s*	*k*	*k*	*k*	*d*
DBA/1	STOLI, B10.Q, BDP	*q*	*q*	*q*	*q*	*q*	*q*

*The R designates a recombinant haplotype, in this case between the H-2^a and H-2^b types. Gene contribution from the *a* strain is shown in yellow and from the *b* strain in red.

Table 8-1
Kuby IMMUNOLOGY, Sixth Edition

Mice in immunology studies

H-2 haplotypes

Progeny of two different inbred strains (F1 generation) are heterozygous at all genetic loci, including MHC

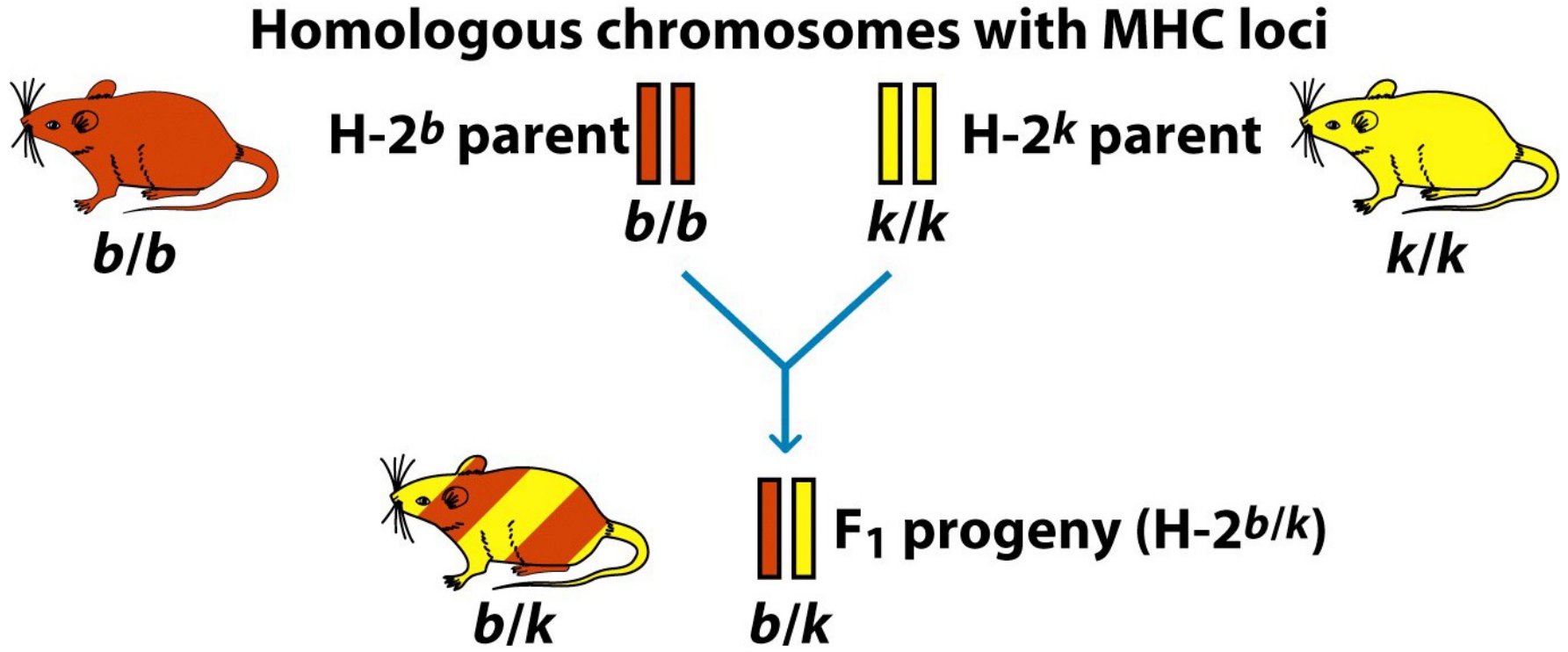


Figure 8-2a
Kuby IMMUNOLOGY, Sixth Edition

Mice in immunology studies

Grafts

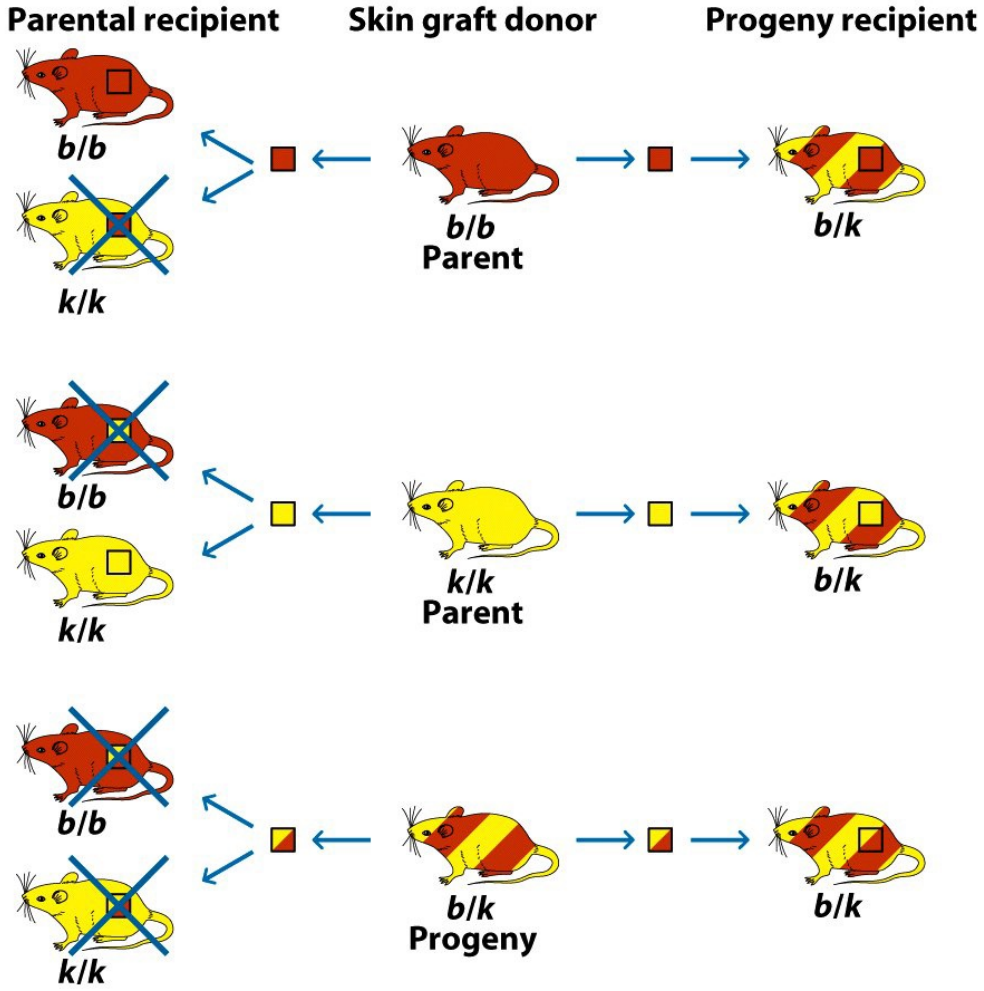


Figure 8-2b
Kuby IMMUNOLOGY, Sixth Edition

Transplants (grafts) require MHC identity

McDevitt's and Gorer & Snell's experiments mapping the genetics of transplantation led to the discovery of the MHC

Graft Nomenclature

- Isograft: Graft from one individual to itself
- Syngeneic: Graft between identical individuals
- Allograft: Graft between individuals of different strain
- Xenograft: Graft between different species

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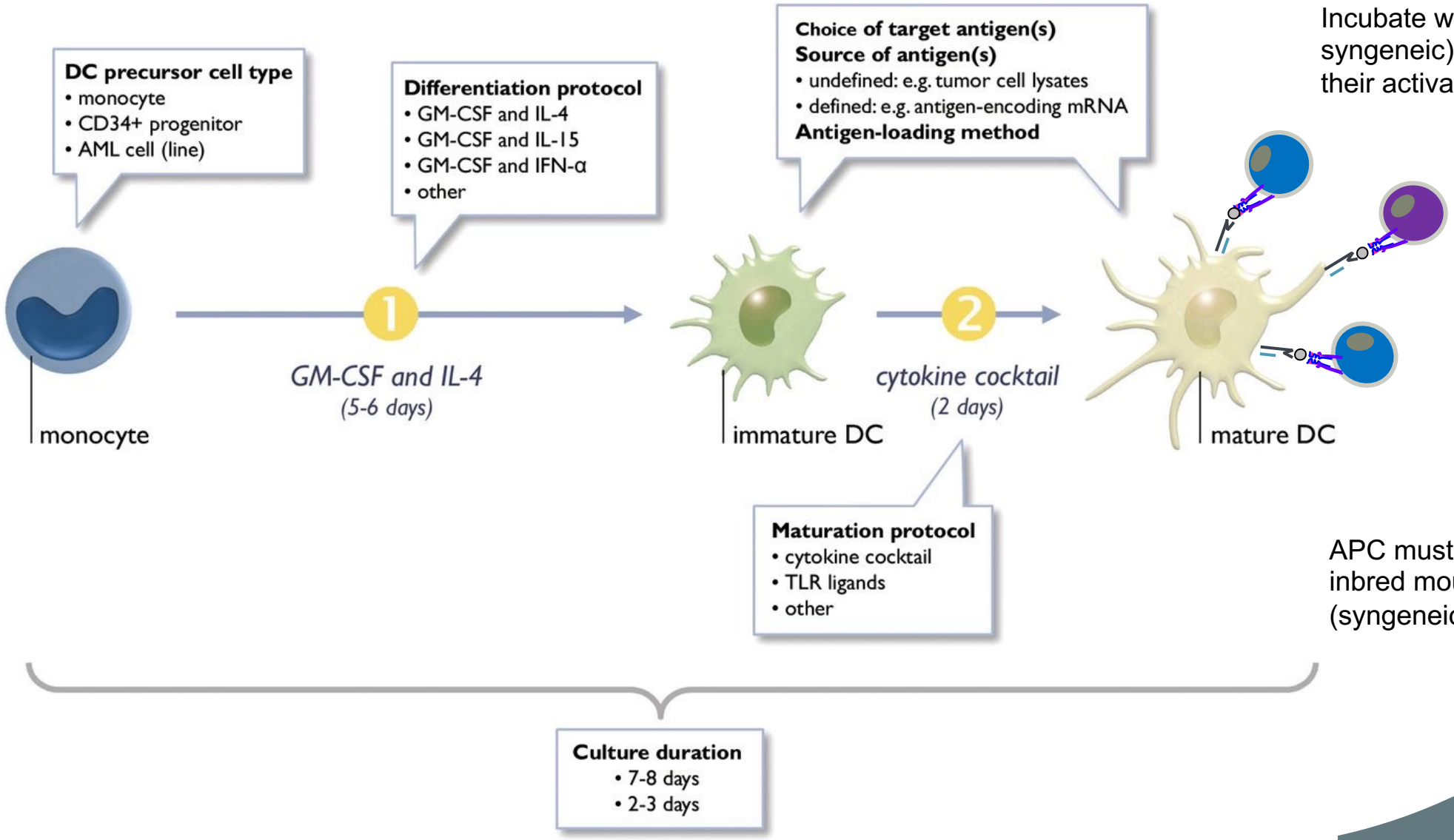
In vitro generation of monocyte derived dendritic cells



Anguille S et al., Pharmacological Reviews 2015, 67 (4) 731-753

Incubate with autologous (or syngeneic) T cells and test their activation

APC must be from same inbred mouse strain (syngeneic) as primed T cell

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Transfer of protective immunity (humoral immunity)

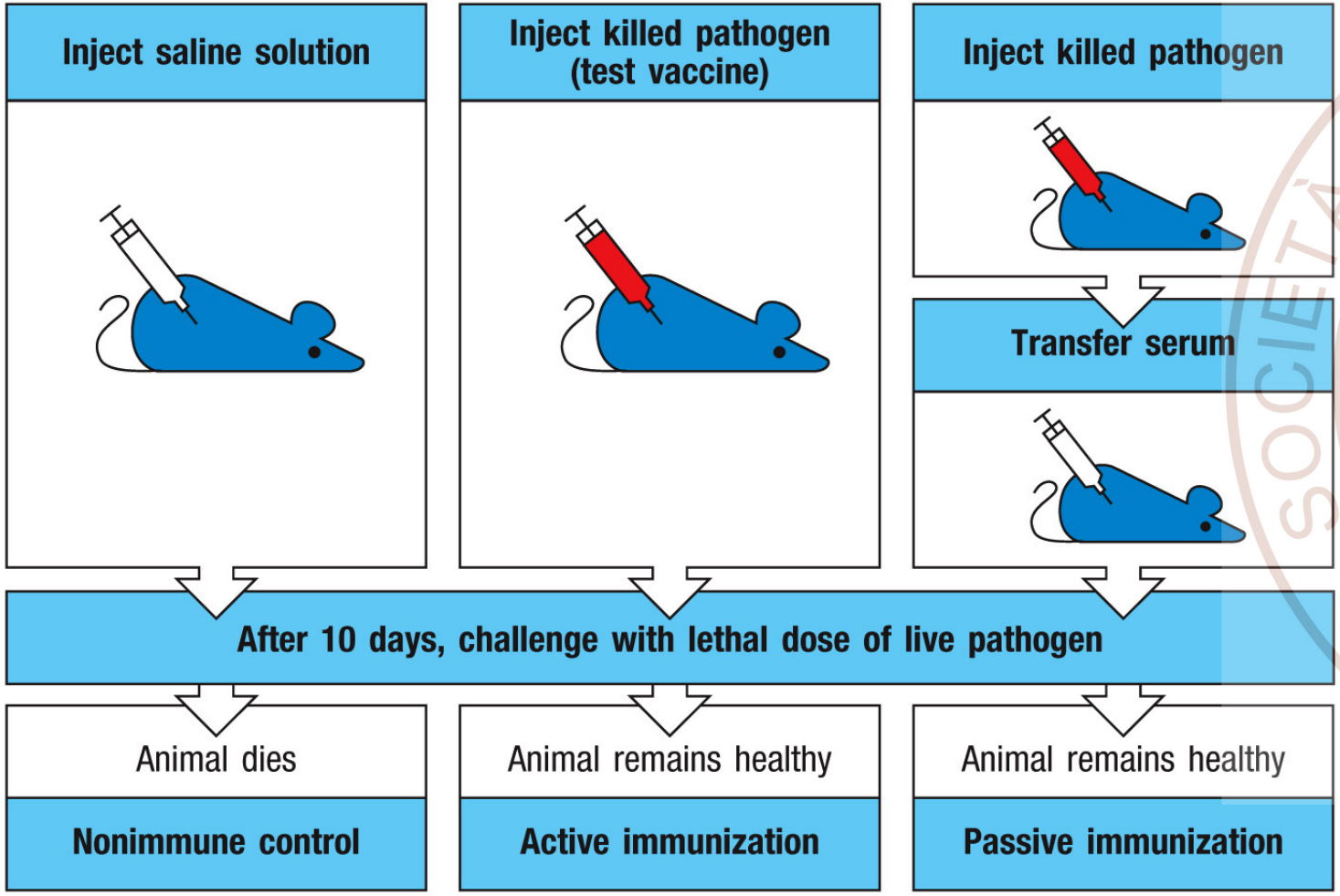
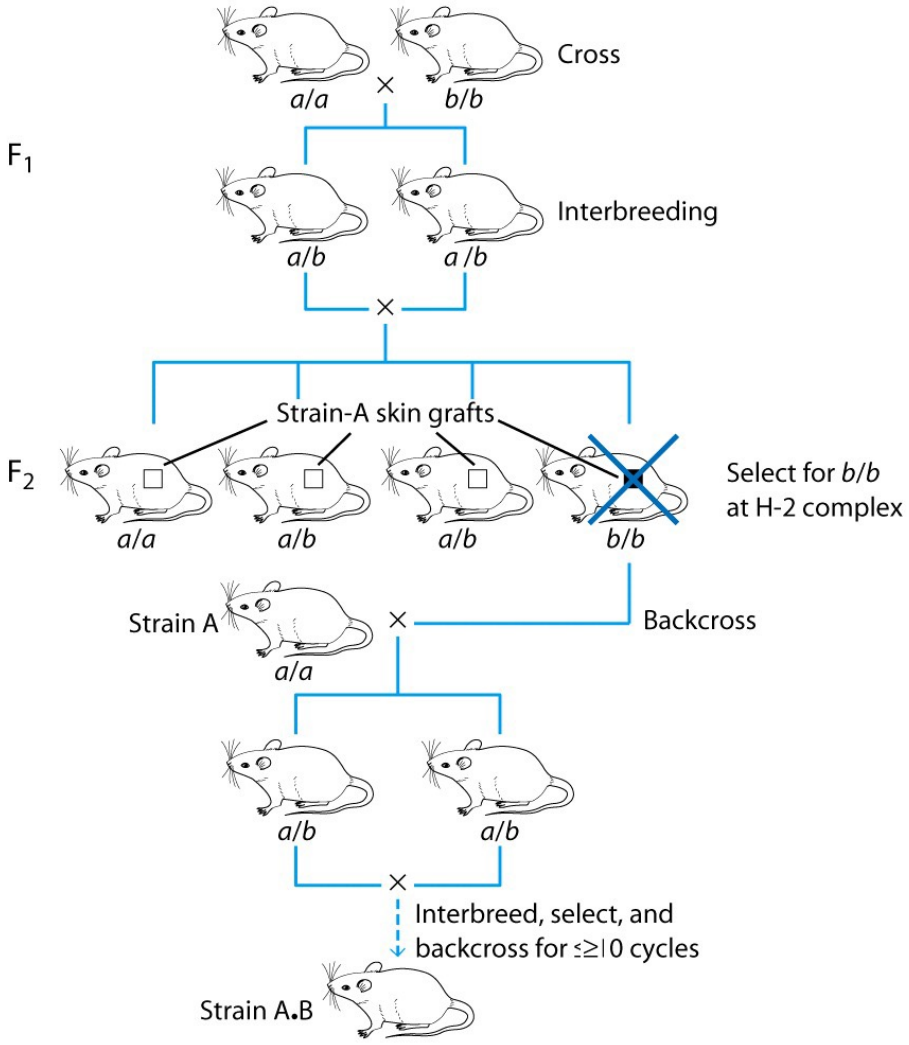


Figure A.40 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Mice in immunology studies

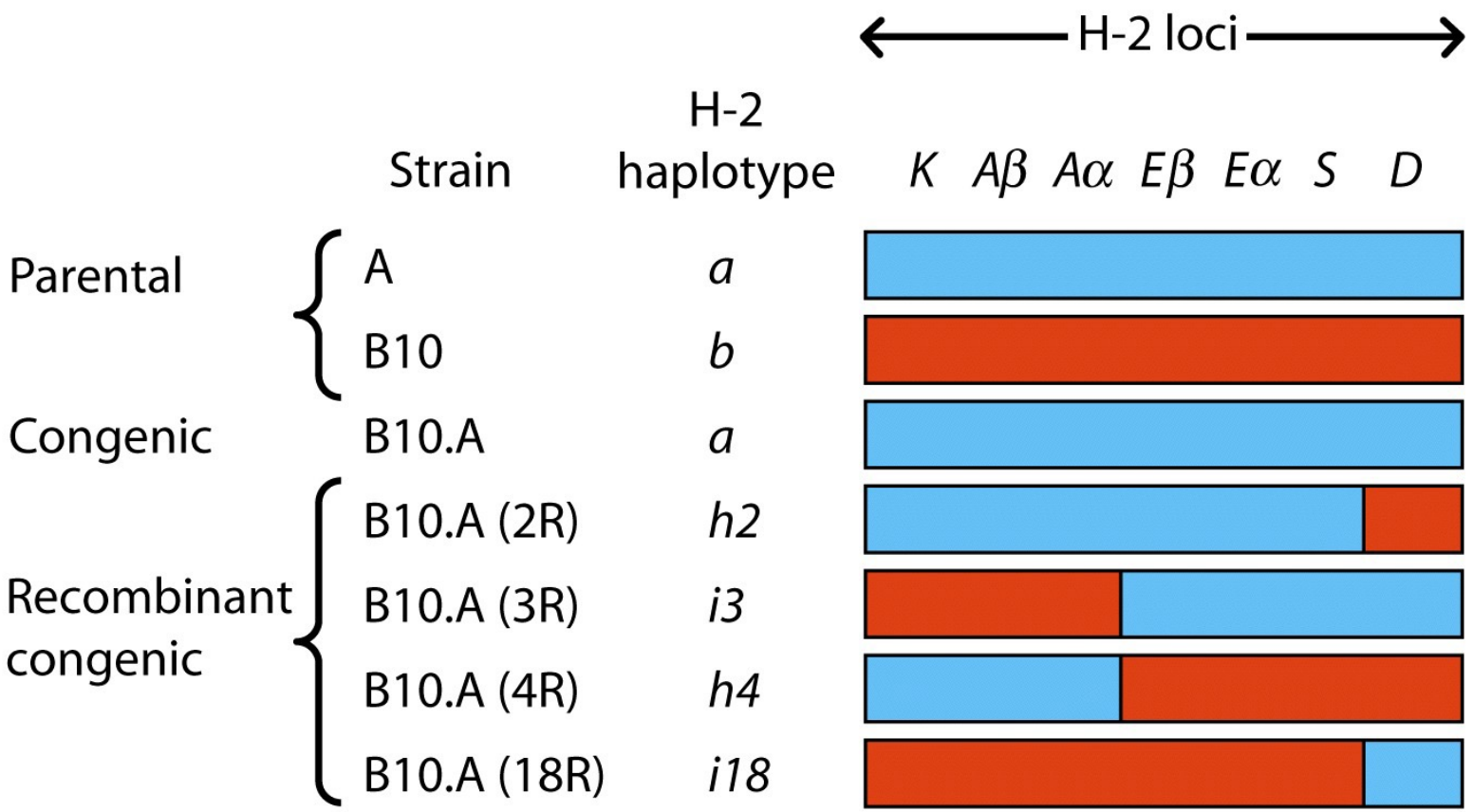
Congenic mice



- “Congenic” mice are genetically identical except for a small genomic region.
- F1 hybrid between inbred strains
- F2 progeny tested for ability to reject skin graft from A mice Selected progeny backcrossed to strain A mouse
- Progeny of backcross tested for ability to reject skin graft from A mice
- Repeat cycle >10 times
- Congenic strain containing all loci from strain A except MHC which is from strain B
- Recombinant congenic strains have part of MHC locus from one parental strain and part from another.

Mice in immunology studies

Congenic mice



"Congenic" mice are genetically identical except for a small genomic region.
Meiotic recombination within the MHC locus can generate congenic mice with portions of the MHC locus derived from each parent ➠ recombinant congenic

Mice in immunology studies

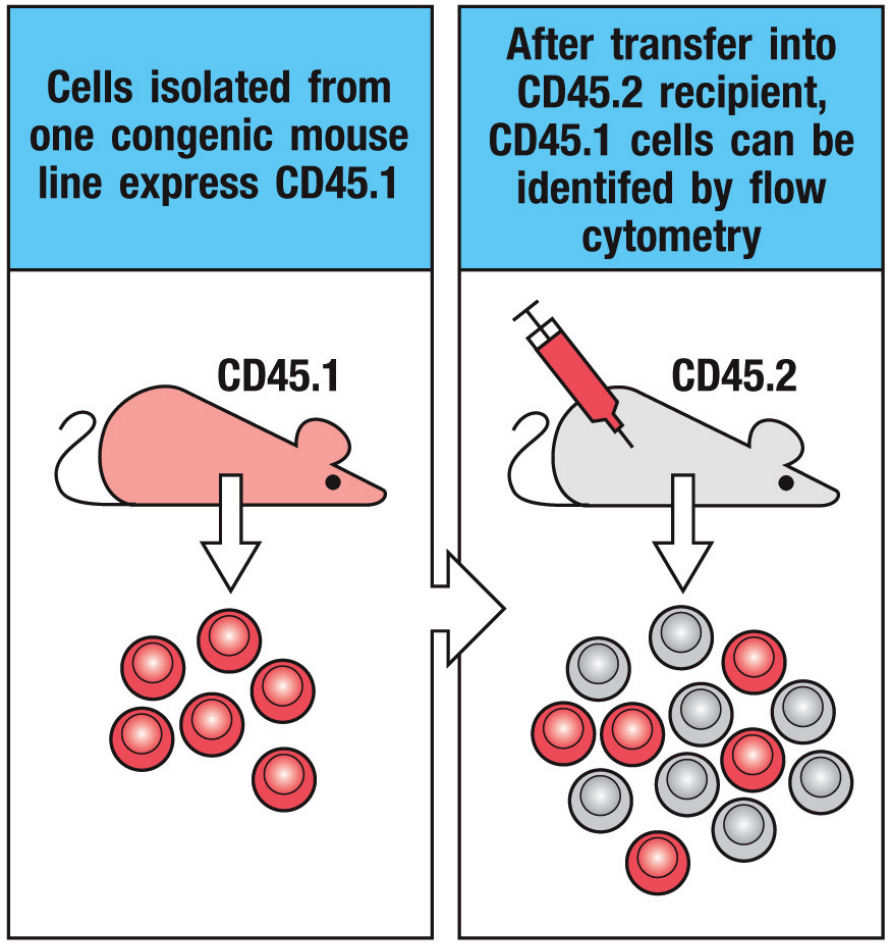


Figure A.41 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Adoptive-acquired immunity: transfer of congenitally marked cells

Hematopoietic cells can be transferred between genetically identical (or nearly identical) mice. The transferred cells, usually a minority population in the recipient, are identified based on expression of an allelic variant of an abundant cell-surface receptor.

One common receptor used for this purpose is CD45, which has two alleles that can be distinguished by allele-specific antibodies.

When cells from a CD45.1+ mouse are transferred into mice of the identical strain (save for their expression of CD45.2), the donor-cell population can easily be identified by antibody staining followed by flow cytometry or immunofluorescence microscopy.

Mice in immunology studies

In vivo depletion of selected immune populations

Transient:

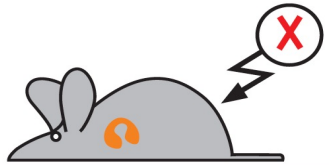
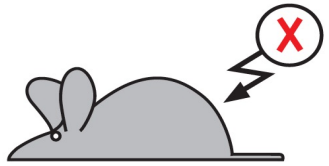
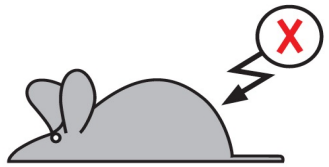
- Irradiation
- Metronomic chemotherapy
- Repeated administration of mAb

Permanent:

- Spontaneous mutation in immune related genes
- Deletion of immune related genes

Mice in immunology studies

Radiation bone marrow chimeras

	Operation	Irradiation	Reconstitution	Induction of cell-mediated immunity
1	Sham thymectomy		Bone marrow	++
2	Thymectomy		Bone marrow	–
3	Thymectomy		Bone marrow + adult lymphocytes	++

X-irradiation (X) destroys the ability of host lymphocytes to mount a cellular immune response, but the stem cells in injected bone marrow can become immunocompetent and restore the response (1) unless the thymus is removed (2), in which case only already immunocompetent lymphocytes are effective (3). Incidentally, the bone marrow stem cells also restore the levels of other formed elements of the blood (red cells, platelets, neutrophils, monocytes) that otherwise fall dramatically after X-irradiation.

Mice in immunology studies

Mice with spontaneous mutations in immune related genes

- In 1960s, a mutation of thymus dysgenesis in mice was discovered, and these mice were called ***nude mice***.
- In 1983, severe combined immunodeficiency (SCID) mice were reported, which were more immunodeficient than nude mice. In ***SCID mice***, there are defects in T- and B-lymphocytes, but the function of natural killer (NK) cells and macrophages is normal.
- In 1992, an extremely immunodeficient strain of mice was reported by the Jackson Laboratory, which was called nonobese diabetic/SCID (NOD/SCID) mouse. ***NOD/SCID mice*** not only lacked functional lymphoid cells similar to SCID mice, but also showed reduced activation of NK cells and had less mature macrophage population.
- All these mice allowed to better characterize the immune system and were defined as ***immunocompromised models*** or xenograft models, which were used to transplant heterologous cells or tissues to establish models systems.

Mice in immunology studies

NUDE MICE

Immunological deficiencies:

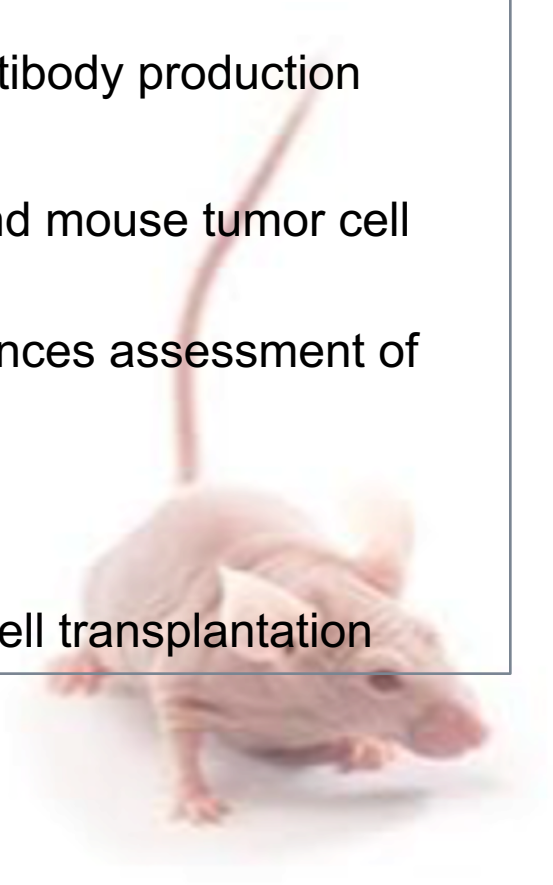
- T cell deficient
- impaired T-dependent antibody production

Applications:

- Engraftment of human and mouse tumor cell lines
- Hairless phenotype enhances assessment of tumor growth

Considerations

- Innate immunity intact
- Not suitable for primary cell transplantation



- Mutation in a recessive gene on chromosome 11, *Foxn1*^{nu}, a developmentally regulated transcription factor selectively expressed in skin and thymic epithelia.
- Mice homozygous for this trait (nu/nu) are hairless and have a vestigial thymus.
- Originally considered as a model of DiGeorge syndrome.
- More recently, the human equivalent has been described, contributing to unravel important issues of the T-cell ontogeny in humans.
- A mutation in this gene in humans has been correlated with T-cell immunodeficiency, the skin disorder congenital alopecia, and nail dystrophy.

Mice in immunology studies

DiGeorge syndrome (DGS)

Common congenital disorder characterized by neural-crest-related developmental defects, associated with deletions of chromosome 22q11.2, where the T-box 1 (TBX1) gene has been mapped.
T-box genes encode transcription factors involved in the regulation of developmental processes.

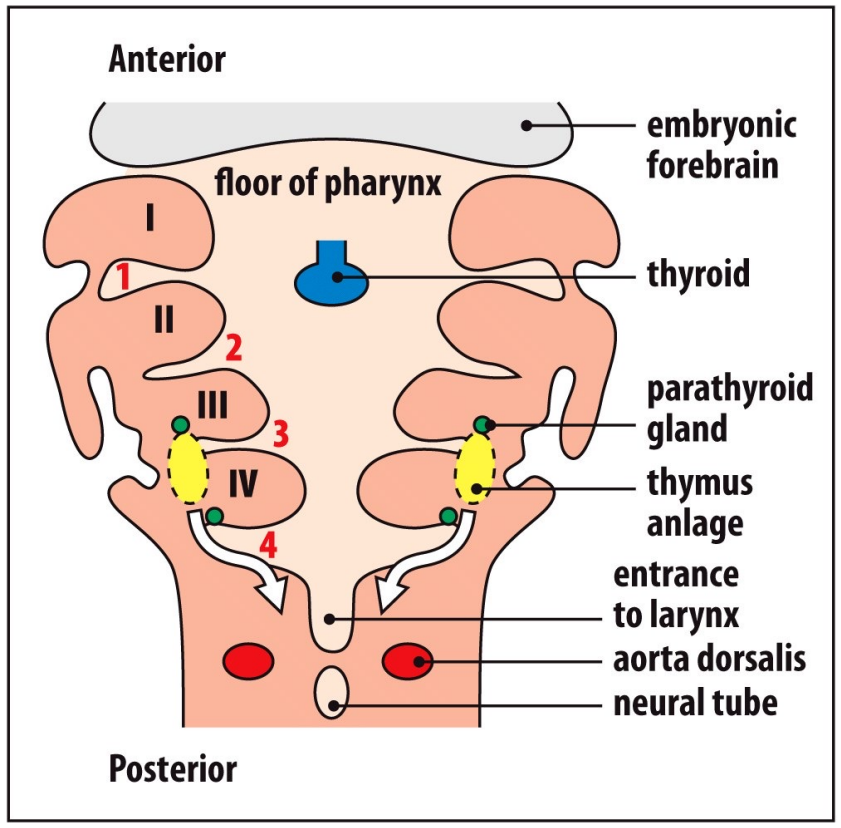


Figure 9.1 Case Studies in Immunology, 6ed. (© Garland Science 2012)

A thymus anlage (yellow) arises from the third pharyngeal pouch on each side, in close proximity to the parathyroid glands. As the embryo develops, the two anlagen come together to form a single organ. The correct segmentation of the embryonic pharynx, which is under the control of the transcription factor Tbx1 , is required for the correct development of the thymus and other organs and structures that develop from this region.

Mice in immunology studies

SCID (Severe combined immune deficiency) mice

Immunological deficiencies:

- T cell deficient
- B cell deficient

Applications:

- Engraftment of human and mouse cells and tissues
- Can be turned into a model of the human immune system when injected with human cells or tissues
- Valuable tool to study immunodeficiency and differentiation process of bone marrow stem cells

Considerations:

- Must be housed in a germ-free environment
- Spontaneous generation of T and B cells during aging (known as leakiness); strain differences in leakiness (B6- and BALB/c- > C3H- >> NOD-scid mice)
- Since Prkdc protein is involved in DNA repair, scid mutants are radiation-sensitive and therefore may not be suitable hosts if they need to be irradiated;
- C.B-17-scid mice retain normal NK and myeloid cell function and fail to support long-term engraftment of the human immune cells that are critical for developing small animal models for human infectious diseases such as HIV.

Homozygous for the $Prkdc^{scid}$ spontaneous mutation (on chromosome 16) responsible for deficient activity of the ***catalytic subunit of the DNA dependent protein kinase*** involved in DNA non-homologous end joining required for double-strand break repair and V(D)J recombination. Mutation initially characterized in a BALB/c congenic strain called C.B-17. In humans: Immunodeficiency 26 (SCID).

Mice in immunology studies

NOD (non obese diabetic) mice

Originally developed in Japan during the selection of a cataract-prone strain. NOD mice harbour ***defects in multiple safety nets***, thereby allowing autoimmunity to occur spontaneously. They spontaneously develop type 1 diabetes and are also prone to developing other autoimmune syndromes.

Immunological deficiencies:

- Absence of circulating complement (devoid of functional C5)
- Defective Natural Killer (NK) cells
- Differentiation and functional deficits in macrophages and antigen presenting cells (APCs)
- Defective expression of CTLA-4 (no T cell inhibition)
- Defective IL2 production (no T reg survival)

Applications:

- Most commonly used mice to model insulin-dependent (Type I) diabetes;
- Evaluation of the role of central and peripheral tolerance in autoimmunity;
- Background of choice in the development of severe immunodeficient mice.
- Considerations:
- The reduced NK cell function in NOD mice potentially improves engraftment of human immune cells
- Macrophages in the bone marrow of NOD mice express a variant of the signal-regulatory protein alpha (Sirpα) that has a higher affinity for human hematopoietic stem cells

Mice in immunology studies

IMMUNODEFICIENT MICE: THE NOD CONNECTION

Two distinct research goals drove the development of NOD immunodeficient models:

- The development of a mouse that could be used to uncover which immune cells actually drive the development of autoimmune diabetes in NOD mice.
- NOD-scid mice, because they are B and T cell-deficient, do not develop diabetes. As a result, they are the perfect hosts for the adoptive transfer of T and B cells from diabetic NOD mice to investigate their roles in the development of autoimmune (Type 1) diabetes.
- The development of a mouse that would support long-term engraftment of human cells and tissues for studies related to human infectious disease and cancer.

Mice in immunology studies

NOD-scid mice

Immunological deficiencies:

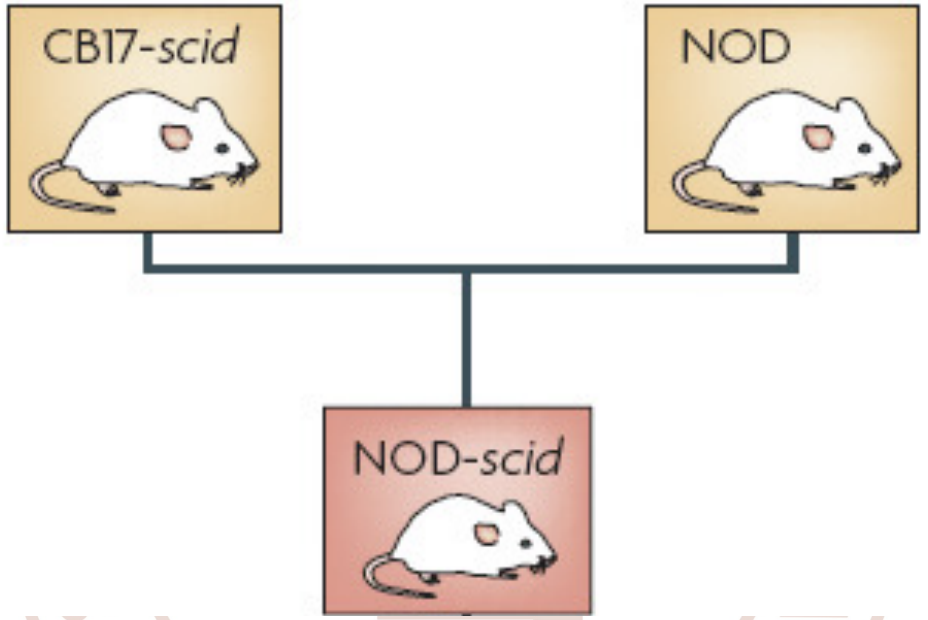
- T and B cell deficient
- Reduced innate immunity

Applications:

- Engraftment of human and mouse cells and tissues
- Adoptive transfer recipient for study of type 1 diabetes

Considerations:

- Short life span (thymic lymphoma by ~ 9 months)
- Scid side effects: increased sensitivity to radiation and genotoxic drugs
- Significantly less scid leakiness compared to other backgrounds



NOD mice have inherent immune deficiencies that enhances the overall immune deficiency of NOD-scid mice in comparison to C.B-17-scid or C57BL/6-scid mice.