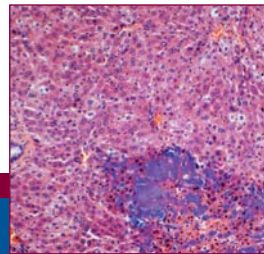
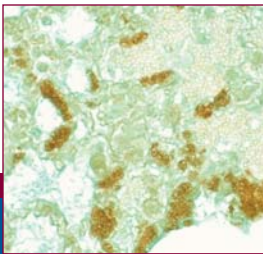


# Infectious Disease Research and the Laboratory Mouse

*A Jackson Laboratory Resource Manual*



This Resource Manual highlights the role of the mouse in infectious disease research, focusing especially on HIV(AIDS) and Category A bioterrorism agents anthrax, botulism, Ebola, plague, smallpox, and tularemia. The Manual includes the following:

- brief descriptions of these diseases,
- summaries of selected studies that have used the mouse to characterize the pathology and immune responses to and develop therapies and vaccines for these diseases, and
- descriptions of selected JAX® Mice models that have been used in the past and that are promising for future research of these diseases.

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## Introduction

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Worldwide, over a third of the people who die each year succumb to infectious diseases such as malaria, tuberculosis, and human immunodeficiency virus/acquired immune deficiency syndrome (HIV)/AIDS (Buer and Balling 2003). The focus of the 2004 World Health Report by the World Health Organization is HIV(AIDS). Since the September 11 attacks on the World Trade Center in New York in 2001, the possibility of bioterrorist attacks has fostered a great deal of research into developing treatments and vaccines for infectious diseases such as anthrax, botulism, Ebola, smallpox, and tularemia. More than ever, the physiology of, pathology caused by, and immune responses to those agents must be thoroughly understood. For this, there is no substitute for *in vivo* experiments with animal models, and, in many cases, there is no better model than the mouse (Buer and Balling 2003; Elkins *et al.* 2003). Indeed, searches through Pub Med ([www.ncbi.nlm.nih.gov/pubmed](http://www.ncbi.nlm.nih.gov/pubmed)) reveal literally thousands of studies using mice to research infectious diseases. Following are some of the reasons why:

- The mouse is susceptible to many human infectious diseases.
- The differential susceptibility of inbred mouse strains to many infectious diseases can be exploited to identify and characterize the genetic bases of infection and immune response.
- The genetic resources available for the mouse are immense and continually expanding.
- The mouse is relatively inexpensive to maintain and has a high reproductive potential.

This Resource Manual highlights the role of the mouse in infectious disease research, focusing especially on HIV(AIDS) and Category A bioterrorist agents anthrax, botulism, Ebola, plague, smallpox, and tularemia. The Manual includes the following:

- brief descriptions of these diseases,
- summaries of selected studies that have used the mouse to characterize the pathology and immune responses to and develop therapies and vaccines for these diseases, and
- descriptions of selected JAX® Mice models that have been used in the past and that are promising for future research of these diseases.

# HIV(AIDS)

In spite of all the research conducted in the past 25 years, HIV(AIDS) is still the world's most significant health problem. According to the World Health Organization (WHO) the number of adults worldwide infected with HIV has risen from less than a hundred thousand in 1980 to about 40 million ([www.who.int/whr/en](http://www.who.int/whr/en)). The CDC estimates that 850,000 to 950,000 United States residents are infected, and that a quarter of these people do not realize they are infected.

Although antiretroviral therapies have had a major impact on the AIDS epidemics in industrially advanced nations, eradicating HIV-1 is currently thought to be impossible for various reasons, including residual viral

reservoirs in blood and infected tissues, the complicated regimens used to administer antiviral therapies, and the evolution of drug-resistant HIV-1 variants (Nakata *et al.* 2005). New therapies and better models for researching the pathogenesis of HIV are needed.

It is beyond the scope of this manual to summarize the pathology of and the research conducted on HIV/AIDS. Rather, we refer you to two Web sites that provide excellent information, ranging from the very basics to the very technical: 1) the "HIV/AIDS Basics & Prevention" site ([www.thebody.com](http://www.thebody.com)) and 2) the World Health Organization site ([www.who.int/whr/en](http://www.who.int/whr/en)).

## *HIV(AIDS) and the Mouse*

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Although mice are biologically similar to humans, their immune systems are not naturally susceptible to HIV. Therefore, there have been many efforts to either find or construct mouse mutants that can engraft and sustain a human immune system, and thereby be infected with HIV. The most promising of these models are immunodeficient models. The advantages and disadvantages of immunodeficient JAX® Mice models that may be particularly useful for HIV/AIDS research are summarized in the Mouse Models Section (Table 4).

# CDC Bioterrorism Disease Categories

To enable the United States public health system and primary healthcare providers to counteract various biological agents, including pathogens that are rarely seen in the United States and that may be used as bioterrorist weapons, the Emergency Preparedness and Response division of the CDC summarized the major features of these agents and classified them as either A, B, or C, depending on how severe a threat they pose ([www.bt.cdc.gov](http://www.bt.cdc.gov)). This Manual deals only with the six Category A agents: anthrax, botulism, Ebola, plague, smallpox, and tularemia. They are classified as Category A agents because they can be easily

disseminated and transmitted from person to person, result in high mortality rates, can have a major impact on public health, may cause public panic and social disruption, and require special action for public health preparedness.

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## Anthrax

### *General Description and Pathology*

Anthrax is an infectious disease caused by the Gram-positive bacterium *Bacillus anthracis*. Its symptoms depend on the route of infection. Most infected people are infected by cutaneous anthrax, which is rarely fatal. In contrast, inhalation anthrax is almost always fatal, and gastrointestinal anthrax is fatal 25% to 60% of the time. Symptoms of inhalation anthrax may initially resemble a common cold, but, after several days, progress to severe breathing problems and shock. Gastrointestinal anthrax is characterized by an acute inflammation of the intestinal tract, nausea, loss of appetite, vomiting, and fever, followed by abdominal pain, vomiting of blood, and severe diarrhea. Once *B. anthracis* is inhaled, its phagocytosis and transport to regional lymph nodes by macrophages and dendritic cells appears to be necessary to establish an infection (Pickering *et al.* 2004). Yearly, between 20,000 and 100,000 people worldwide

contract anthrax, mostly from contact with infected animals, including insects (Roberts *et al.* 1998; CDC, [www.bt.cdc.gov](http://www.bt.cdc.gov)).

The symptoms of systemic anthrax are caused mostly through the actions of three polypeptides produced by *B. anthracis*: protective antigen (PA), edema factor (EF), and lethal factor (LF), collectively called anthrax toxin or lethal toxin (LT). LT is particularly poisonous to macrophages (Moayeri *et al.* 2004; Roberts *et al.* 1998; CDC, [www.bt.cdc.gov](http://www.bt.cdc.gov)). The pathogenesis of infection by *B. anthracis* and the role played by macrophages is unclear (Cote *et al.* 2004).

Current therapies for anthrax are limited: whereas antimicrobials target replicating bacteria, the bacterial toxins continue to cause a great deal of physiological damage. Novel approaches to combat these cytotoxic effects are needed (Artenstein *et al.* 2004). For detailed information on treatment and vaccines, see the CDC web site ([www.bt.cdc.gov](http://www.bt.cdc.gov)).

## Anthrax and the Mouse

The mouse has been used extensively to research the virulence mechanisms, pathology, and immune response

associated with anthrax infections. Much of the research was conducted by using either JAX® Mice or comparable models.

## Differential strain susceptibilities

Several researchers, including Dietrich (1998), Lyons *et al.* 2004, Roberts *et al.* (1998), and Welkos *et al.* (1986), have characterized the susceptibility of various inbred mouse strains and their macrophages to *B. anthracis* infection and anthrax LT (Table 1). Susceptibility of a strain to infection

by the bacterium does not necessarily correspond to the susceptibility of its macrophages to anthrax LT. For example, the A/J strain is very susceptible to low doses of anthrax spores, yet its macrophages are resistant to anthrax LT.

**Table 1.** Susceptibility of various JAX® Mice strains and their macrophages to *B. anthracis* infection and lethal toxin (superscripts indicate references, listed at the bottom of the table).

Strains	Susceptibility* to LT	Susceptibility to Infection**
A/J	R <sup>1,3</sup>	Sensitive to aerosol infection, <sup>3</sup> and Sterne strain <sup>4</sup>
AKR/J	R <sup>1,3</sup>	na
C57BR/cdJ	na	Resistant to Sterne strain <sup>4</sup>
C57BL/6J	R <sup>1,3</sup>	Resistant to Sterne strain <sup>4</sup>
C58/J	na	Resistant to Sterne strain <sup>4</sup>
DBA/2J	R <sup>1,3</sup>	Sensitive to aerosol infection <sup>3</sup> and Sterne strain <sup>4</sup>
I/LnJ	R <sup>1</sup>	na
PL/J	R <sup>1,3</sup>	na
SM/J	R <sup>1,3</sup>	na
SPRET/EiJ	R <sup>1,3</sup>	na
ST/bj	R <sup>1,3</sup>	na
129P1/ReJ	R <sup>1</sup>	na
129X1/SvJ	R <sup>1</sup>	na
129S6/SvEvTac-Was <sup>tm15bs</sup> /J	R <sup>1,3</sup>	na
129S1/SvImJ	S <sup>1</sup>	na
BALB/cJ	S <sup>1,3</sup>	Resistant to aerosol infection <sup>3</sup> and Sterne strain <sup>4</sup>
B6129XF1	S <sup>1</sup>	na
B6C3F1/J	S <sup>1</sup>	na
C3H/HeJ	S <sup>1,3</sup>	Sensitive to aerosol infection; <sup>3</sup> resistant to Sterne strain <sup>4</sup>
C57L/J	S <sup>1,3</sup>	Resistant to Sterne strain <sup>4</sup>
(C57BL/6J x C3H/HeJ)F1	S <sup>3</sup>	na
CAST/EiJ	S <sup>1,3</sup>	na
CBA/J	S <sup>1,3</sup>	Resistant to Sterne strain <sup>4</sup>
I/LnJ	S <sup>1</sup>	na
PL/J	S <sup>1,3</sup>	na
FVB/NJ	S <sup>1</sup>	na
LP/J	S <sup>1</sup>	na
MRL/MpJ	S <sup>1</sup>	na
NZB/BINJ	S <sup>1</sup>	na
SWR/J	S <sup>1,3</sup>	na

\*R: resistant; S: susceptible  
 \*\*Welkos *et al.* (1986) characterized the susceptibility of ten inbred mouse strains to three forms of *B. anthracis*: 1) a toxigenic encapsulated strain (Vollum 1B), 2) a toxigenic non-encapsulated strain (Sterne), and 3) a nontoxigenic encapsulated strain (Pasteur).  
 na: not available  
<sup>1</sup>Dietrich 1998; <sup>2</sup>Lyons *et al.* 2004; <sup>3</sup>Roberts *et al.* 1998; <sup>4</sup>Welkos *et al.* 1986

## Brief abstracts of selected studies

### Genetic regulation

- A study of numerous JAX® Mice strains revealed that the differential susceptibility of macrophages to LT among inbred mouse strains is controlled by the NACHT, leucine rich repeat and PYD containing 1 (*Nalp1b*) gene, an extremely polymorphic gene in the *Ltxs1* locus on Chr 11. The controlling gene was formerly thought to be *Kiflc*, also a polymorphic gene, in the kinesin-like motor protein of the UNC104 subfamily (Watters *et al.* 2001). The study also revealed that LT-induced macrophage death requires the activation of caspase-1, suggesting that *Nalp1b* directly or indirectly activates caspase-1 in response to LT (Boyden and Dietrich 2006)
- An analysis of a cross between the anthrax-susceptible C3H/HeJ and the anthrax-resistant C57BL/6J strains revealed that anthrax susceptibility to LF in mice is dominant to resistance and is regulated by a single locus, named lethal toxin 1 (*Ltx1*), on Chromosome (Chr) 11 (Roberts *et al.* 1998).
- An analysis of interval-specific recombinant congenic lines carrying various segments of central Chr 11 derived from LT-resistant DBA/2 mice on the LT-sensitive BALB/c background revealed that: 1) anthrax susceptibility in mice is controlled by three linked quantitative trait loci (QTL): lethal toxin 1 (*Ltxs1*, 42–43 cM), lethal toxin 2 (*Ltxs2*, 35–37 cM), and lethal toxin 3 (*Ltxs3*, 45–47 cM); 2) inhibiting nitric oxide 2 (*Nos2*, an attractive *Ltxs3* candidate) *in vivo* partially overrides LT resistance; 3) *Nos2* expression differs significantly between DBA/2 and BALB/c macrophages; and 4) dominant resistance to anthrax requires DBA/2 alleles at all three QTLs (McAllister *et al.* 2003).

### Immune responses

- *Tnfr*-, *Nos2*-, *Tnfa*-, and *Il1r*-deficient mice and controls infected subcutaneously with LD95 of anthrax spores (5 x 10<sup>6</sup>) all succumb at the same frequency. Whereas TNF antibody delays death, TNFR1 has no effect. *Il1r*- and *Nos2*-deficient mice die sooner. Anthrax is more abundant in the injection site of *Tnfa*- and *Nos2*-deficient mice than it is in controls, suggesting that attenuated cellular response increases the rate of disease progression. With the exception of edema and necrosis at the injection site,

internal organs have no pathological changes (Kalns *et al.* 2002a).

- Wild-type mice infected either parenterally or aerogenically with virulent ungerminated *B. anthracis* survive better than do similarly infected macrophage-depleted mice; infected wild-type mice supplemented with cultured macrophages survive better than do controls (Cote *et al.* 2004).
- LT-mediated induction of cytokines Kcno, MCP-1/JE, MIP2, eotaxin (CCL11), and Il1 occur only in mice with LT-sensitive macrophages. LT-mediated death in mice is regulated by multiple genes, including those that control macrophage lysis and cytokine response. LT-mediated death in mice is independent of *Tlr4* function. CAST/Ei mice are uniquely sensitive to LT and may be an economical bioassay for toxin-directed therapeutics (Moayeri *et al.* 2004).
- Cells infected with *B. anthracis* mount an acute cytokine response. Peritoneal macrophages from A/J mice and human dendritic cells produce large amounts of TNFA and IL6. Human dendritic cells also produce IL1B, IL8, and IL12. The sera of A/J mice infected with aerosolyzed *B. anthracis* contains a mixture of Th1 and Th2 cytokines (Pickering *et al.* 2004).

### Pathogenesis

- An analysis of LT toxicity in BALB/cJ and C57BL/6J mice revealed that BALB/cJ mice become terminally ill earlier and with higher frequency than do C57BL/6J mice. LT causes major bone marrow, spleen, and liver damage, pleural edema, and hypoxia in both strains. The effect of LT on the production of 50 different cytokines is examined. Whereas BALB/cJ mice respond to LT by quickly but transiently increasing their production of many cytokines (including KC, MCP-1/JE, IL6, MIP2, G-CSF, GM-CSF, eotaxin, FasL, and IL1B), C57BL/6J mice do not. Mice of neither strain respond by changing their production of TNFA. The researchers conclude that LT kills BALB/cJ and C57BL/6J mice through a hypoxia-mediated non-inflammatory mechanism that is independent of TNFA, FASL, and macrophage sensitivity (Moayeri *et al.* 2003).

## Brief abstracts of selected studies (cont.)

- Pathogenesis and infective doses for pulmonary, subcutaneous, intranasal, and intratracheal *B. anthracis* infections in BALB/c, DBA/2, C3H, 129X1/SvJ, C57BL/6J, and A/J mice are found to depend on the method of infection and to be independent of age and sex (Lyons *et al.* 2004).

### Therapies

- PlyG lysin, a bacteriophage enzyme isolated from the gamma phage of *B. anthracis*, kills germinating spores, vegetative cells, isolates, and various 'cluster' members of *B. anthracis* *in vitro* and *in vivo* in BALB/c mice. The lytic specificity of PlyG can be used to quickly identify *B. anthracis* (Schuch *et al.* 2002).
- Blk57/B6 mice are protected from subcutaneous infections of Sterne strain anthrax spores if they are simultaneously treated with 1.5mg doxycycline/kg body weight. Only 90% survive if they are treated four hours after infection, and none survive when treated 24 hours after infection. Peritoneal macrophages of infected mice produce less TNFA and more IL6 than do macrophages from an RAW 264.7 mouse cell line. These findings suggest that antibiotics are not very effective in fighting an anthrax spore infection (Kalns *et al.* 2002b).
- Chloroquine (CQ), a commonly used anti-malarial agent, gives anthrax-infected murine peritoneal macrophages and BALB/c mice a survival advantage over controls, suggesting that it may improve current anthrax therapies (Artenstein *et al.* 2004).

### Vaccines

- Vaccine made with *B. anthracis* protective antigen (PA) and supplemented with inactivated *B. anthracis* spores totally protects mice and guinea pigs from virulent anthrax infection, suggesting that such vaccines are better than those containing only PA antigens (Brossier *et al.* 2002).
- Administering beta1,3-glucan immune modulators (either PGG-glucan or WGP beta glucan) to BALB/c mice significantly increases their protection against *B. anthracis*, suggesting that it may have similar benefits in humans (Kournikakis *et al.* 2003).
- An assay to measure the serological response of female A/J mice inoculated with a new rPA-based anthrax vaccine may be used for monitoring the consistency of the vaccine in clinical trials (Little *et al.* 2004).
- Immunity to aerosolized *B. anthracis* conferred to A/J mice vaccinated transcutaneously with recombinant protective *B. anthracis* antigen and *E. coli* heat-labile toxin via a patch is comparable to that induced by intramuscular vaccination, suggesting that an anthrax vaccine patch is feasible and should be clinically evaluated (Kenney *et al.* 2004).
- Twelve-week old SCID/bg mice engrafted with peripheral blood lymphocytes from humans immunized with Anthrax Vaccine Adsorbed (AVA) and then vaccinated with anthrax protective antigen and lethal factor produced a significant amount of antigen specific human IgG in serum. The antibodies were diverse, and all were potent inhibitors of anthrax lethal toxin *in vitro*. Single and very small doses of either the AVP-21D9 or AVP-22G12 antibodies fully protected rats from anthrax lethal toxin. Additionally, glycosylated versions of the most potent antibodies fully protected the rats, suggesting that lethal toxin neutralization is not Fc effector mediated. Antibodies like those produced in this study might be effective for either treating or protecting people exposed to anthrax toxins (Sawada-Hirai *et al.* 2004).

# Botulism

## General description and pathology

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Botulism is a neuroparalytic disease caused by a potent neurotoxin produced by *Clostridium botulinum*, an anaerobic, spore-forming bacterium. Depending on how it is transmitted, botulism occurs in three forms: food botulism, usually contracted from improperly preserved foods, wound botulism, usually contracted from spores in the soil, and infant (enteric) botulism, usually contracted by children under six months old from spores that germinate in the gastrointestinal tract ([www.bt.cdc.gov](http://www.bt.cdc.gov)). The excellent review of infant botulism by Arnon (1987) summarizes the worldwide reports of the disease since its recognition in 1976, selected case histories (including typical and atypical symptoms), intestinal pathophysiology, the relationships

among diet, intestinal flora, and botulism susceptibility, clinical spectra and new diagnostic techniques, and the various groups of *Clostridium* and their poisons.

Botulism paralyzes the nervous system, always starting with the cranial nerves and descending symmetrically through other motor and autonomic nerves. Symptoms include double and blurred vision, drooping eyelids, slurred speech, difficulty swallowing, dry mouth, and muscle weakness. If untreated, it may paralyze respiratory muscles, arms, and legs. It may be arrested if *botulinum* antitoxin is administered early. Recovery takes months, but fatigue and shortness of breath may last for years. About 5% of affected people die ([www.bt.cdc.gov](http://www.bt.cdc.gov)).

## Botulism and the mouse

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The mouse has been used, though not extensively, to research the virulence mechanisms, pathology, and immune response associated with botulism infections.

## Brief abstracts of selected studies

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### Infant botulism

As with humans, mice and rats are susceptible to enteric botulism only in infancy: *C. botulinum* spores administered in the guts of mice and rats multiply only in 7 to 13 day old individuals (Sugiyama and Mills 1978; Sugiyama 1979). In contrast, adult mice and rats are resistant, even when infected intragastrically with  $10^6$  or more spores. Burr and Sugiyama (1982) demonstrated that this resistance is due to the large diversity and numbers of intestinal flora in the adult rodent gut: adult mice treated with antibiotics that kill intestinal flora are very susceptible to infections of as few as 10 spores. They suggest that even the occasional case of enteric botulism in adult humans is due to a low number and diversity of intestinal flora (perhaps as a result of antibiotics taken to kill other infections).

### Vaccines

- Whereas mice vaccinated intramuscularly with serotype A gene products are protected only against an intraperitoneal challenge of serotype A neurotoxin, those vaccinated with serotype A and B gene products are protected from intraperitoneal challenges of serotype A and B toxins respectively (Smith 1998).
- Although BTBH-N1, a neutralizing mouse monoclonal antibody developed against *C. botulinum* neurotoxin serotype B (BoNT/B), does not protect mice challenged with 100 units of BoNT/B, it significantly prolongs time to death in a dose dependent manner, suggesting that it could be further developed as an effective botulism therapy (Yang *et al.* 2004).

# Ebola

## *General Description and Pathology*

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Ebola was named after a river in the Democratic Republic of the Congo, where it was first recognized in 1976. It is a highly infectious, often-fatal viral disease in humans and non-human primates. It is one of a host of diseases called viral hemorrhagic fevers (VHFs). The disease-causing pathogen is a member of a family of lipid-coated RNA viruses called the *Filoviridae*. Like other *Filoviridae*, Ebola depends on an animal host, its natural reservoir, which in Ebola's case, is unknown. Outbreaks have occurred sporadically and unpredictably in various regions of Africa ([www.bt.cdc.gov](http://www.bt.cdc.gov)).

There are four identified subtypes: three, Ebola-Zaire (EBO-Z), Ebola-Sudan, and Ebola-Ivory Coast, cause disease in humans; the fourth, Ebola-Reston, has caused disease only in nonhuman primates ([www.bt.cdc.gov](http://www.bt.cdc.gov)). EBO-Z is fatal up to 90% of the time (Gibb *et al.* 2001). Humans contract EBO-Z after contacting infected hosts and other humans. Onset is abrupt and characterized by fever, headache, joint and muscle aches, sore throat, and weakness, diarrhea, vomiting, and stomach pain. Some patients develop a rash, red eyes, hiccups, and internal and external bleeding. There is currently no treatment, cure, or vaccine for Ebola ([www.bt.cdc.gov](http://www.bt.cdc.gov)).

## *Ebola and the Mouse*

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Since it was recognized in 1976 and until recently, EBO-Z had been studied primarily in non-human primates and guinea pigs. Although the virus infected young mice, it did not infect adults. Nevertheless, there was considerable impetus for developing the mouse as an Ebola model. By serially passing EBO-Z virus in progressively older suckling mice, Bray *et al.* (1999) obtained a virus that was lethal for

mature, immunocompetent BALB/c and C57BL/6 inbred and ICR (CD-1) outbred mice. The pathology of these mice when infected with "mouse-adapted EBO-Z" resembles that in EBO-Z-infected primates.

Following are summaries of selected Ebola research conducted on either JAX® Mice or comparable models.

## Brief abstracts of selected studies

### Immune responses

- BALB/c mice infected subcutaneously with live mouse-adapted EBO-Z survive infection and generate high levels of serum anti-IgG. Passive transfer of this serum to naïve mice, including BALB/c *scid* mice, protects up to 100% of them against lethal infection (protection correlates with the amount of IgG). The antibodies protect against lethal infection by either suppressing or delaying the growth of the virus, suggesting that other immune components are not needed (Gupta *et al.* 2001).
- BALB/cJ mice immunized intravenously with L(EV) (liposome-encapsulated irradiated EBO-Z containing all of the native EBO-Z proteins) are completely protected when later challenged with mouse-adapted EBO-Z. Only 55% of mice immunized with non-encapsulated irradiated virus (EV) survive challenge, and all become ill. Treatment with anti-CD4 antibodies either before or during immunization with L(EV) eliminates protection. Antigen-specific CD4 cells secrete IFNG. Similar L(EV) immunization is not successful in monkeys (Rao *et al.* 2002).
- Long term protection conferred to BALB/c mice initially infected subcutaneously with mouse-adapted EBO-Z is associated with an attenuated inflammatory response and early production of antiviral cytokines, particularly IFNA (Mahanty *et al.* 2003).
- Recombinant vaccine vectors that express proteins to induce high levels of Ebola-specific T cells are used to 1) identify the Ebola antigens that CD8 T cells recognize, 2) construct a detailed map of  $H2^b$  and  $H2^a$  restricted CD8 T cell epitopes from nucleoprotein (NP), and 3) perform a comparative analysis of several heterologous prime/boost vaccine strategies (Simmons *et al.* 2004).
- Experiments using CD8 T-cell-deficient mice (B6.129P2-*Tcrb<sup>tm1Mom</sup>/J*), B cell-deficient mice (B6.129S2-*Igh-6<sup>tm1Cgn</sup>/J*), and CD4 T cell-deficient mice (B6.129S2-*Cd4<sup>tm1Mak</sup>/J*) revealed that whereas CD8 cells help protect against acute Ebola infection, CD4 cells and antibodies are required for long-term protection. Under certain immunodeficiency conditions, the virus can persist, and loss of primed CD4 T cells accelerates the course of persistent infections (Gupta *et al.* 2004).
- Whereas *Fas*-deficient mice (B6.MRL-*Fas<sup>bp</sup>/J*), 90% of *Ifng*-deficient mice (B6.129S7-*Ifng<sup>tm1Ts</sup>/J*), and all C57BL/6J controls survive sub-cutaneous infections with mouse-adapted Ebola, perforin-deficient mice (C57BL/6-*Prf1<sup>tm1Sdz</sup>/J*) all die. They fail to clear the infections even though they develop normal levels of neutralizing anti-Ebola antibodies, 5 to 10 times more IFNG, and 2 to 4 times more Ebola-specific CD8 cells than do controls. These findings suggest that clearing Ebola is perforin-rather than IFNG-dependent. Other than in a few isolated instances, perforin had traditionally been associated with clearing only noncytopathic viruses (Gupta *et al.* 2005).

### Therapies

- EBO-Z viral replication is inhibited *in vitro* in a dose-dependent manner by a series of nine nucleoside analogue inhibitors of S-adenosylhomocysteine hydrolase, an important target for antiviral drug development and the first compound demonstrated to cure animals from an otherwise lethal virus infection (Huggins *et al.* 1999).
- A single early dose of 3-deazaneplanocin A, an analog of adenosine, prevents illness and death in EBO-Z-infected mice. The drug appears to reverse Ebola's ability to suppress innate antiviral mechanisms of the type I interferon response, thereby restricting viral dissemination (Bray *et al.* 2002).

### Vaccines

- C57BL/6 mice vaccinated with Venezuelan equine encephalitis virus replicons encoding the Ebola virus nucleoprotein (NP) survive a lethal Ebola infection. The vaccination induces the production of NP-specific antibodies and CD8(+) cells. Whereas passive transfer of polyclonal NP-specific antiserum does not protect unvaccinated mice, adoptive transfer of NP-specific CD8(+) cells does. Thus, the development of Ebola vaccines should consider that protective cellular immune responses may be required for optimum protection (Hart and Wilson 2001).
- BALB/c mice vaccinated with either mouse-adapted EBO-Z or Venezuelan equine encephalitis virus (VEEV) DNA are completely protected against later infections with the diseases (Riemenschneider *et al.* 2003).

# Plague

## General Description and Pathology

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Plague is caused by the bacterium *Yersinia pestis*. It occurs in two basic forms: bubonic and pneumonic. Bubonic plague, so named because it causes massive enlargements, “buboes,” of lymph nodes, generally results from the bite of an infected flea. Sometimes buboes do not develop, in which case the plague is termed septicemic. In either case, the infection causes fever, headache, malaise, and gastrointestinal problems. If detected early, before symptoms develop, plague can be treated; if untreated, it can progress to the lung and develop into secondary pneumonic plague.

Plague can also be transmitted from either an infected person or other animal through the air, in which case it develops into primary pneumonic plague, the most feared and virulent form of the disease, and the form bioterrorists would likely use (Alpar *et al.* 2001; [www.bt.cdc.gov](http://www.bt.cdc.gov)). Pneumonic plague is particularly feared because infection can go undetected until symptoms develop (Alpar *et al.* 2001).

Outbreaks of plague are rare: 5 to 15 cases, mostly bubonic, occur each year, scattered throughout rural and semi-rural western United States. Pneumonic plague is uncommon, but outbreaks could occur, either naturally or as a result of bioterrorism ([www.bt.cdc.gov](http://www.bt.cdc.gov)).

Plague vaccines were formerly made with killed whole cell preparations of *Y. pestis*. In mouse models, such vaccines confer moderate protection against bubonic plague but relatively little against pneumonic plague (Hill *et al.* 2003; Leary *et al.* 1999). The results are also less than ideal in humans: as many as 25% of vaccinated people experience side effects, some quite serious. Consequently, whole-cell vaccines are no longer used in the United States. Instead, vaccines are constructed with purified *Y. pestis*-specific V and F1 antigens. These vaccines provide better protection, especially when administered together, and produce few and relatively mild side effects (Hill *et al.* 2003; Leary *et al.* 1999). See the CDC web site ([www.bt.cdc.gov](http://www.bt.cdc.gov)) for additional information on *Yersinia* treatments and vaccines.

## Plague and the Mouse

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The mouse has been used extensively to determine the pathogenesis of, immune response to, and efficacy of various drugs and vaccines against *Y. pestis*. Following are brief

descriptions of some of the results of those studies in the last ten years. Most efforts have been to improve currently used vaccines.

## Brief abstracts of selected studies

### Immune responses

- The *Y. pestis* V antigen inhibits the migration of neutrophils to the site of a plague infection. Sera from Swiss Webster mice surviving such an infection inactivates the inhibitory action of the antigen (Welkos *et al.* 1998).
- Severe combined immunodeficient/beige (SCID/Bge) mice with the *Prkdc<sup>scid</sup>* and *Lyst<sup>b<sup>g</sup></sup>* mutations and reconstituted with hyperimmune BALB/c lymphocytes are protected against inhaled and systemic *Yersinia* infections, demonstrating the ability of passive and adoptive immunity to protect against plague (Green *et al.* 1999).
- After antibiotic treatment, Swiss-Webster mice (Hsd:ND4) surviving a lethal *Yersinia* aerosol challenge mount a humoral immune response, primarily against F1, V, YopH, YopM, YopD, and Pla *Y. pestis* antigens, suggesting that only some virulence factors are expressed and/or immunogenic during infection. These results may help researchers select potential vaccine candidates and improve serologic diagnostic assays (Benner *et al.* 1999).
- Experiments with BALB/c mice revealed that protection against *Y. pestis* correlates significantly ( $P < 0.05$ ) with the production of the IgG1 subclass of antibodies directed against the V and F1 antigens. Titers of IgG1 determined for 90%, 50%, and 10% protection were determined and may predict vaccine efficacy in humans (Williamson *et al.* 1999).
- Whereas 6- to 8-week old C57BL/6J and B6.CB17-*Prkdc<sup>scid</sup>/SzJ* mice succumb when infected with a conditionally virulent *Y. pestis* KIM5 mutant, they die when infected with a yopM deletion mutant. Populations of B6 immune system cells are not differentially affected by either *Y. pestis* strain, except that the KIM5 infection causes a significant and early decrease in their blood, spleen, and liver NK cells. NK cells and macrophages isolated on day 2 from livers and spleens of mice infected with either *Y. pestis* strain contain comparable levels of cytokine mRNA (IL1B, IL12, IL15, IL18, and TNFA in macrophages; IFNG in NK cells). By day 4, cells infected with the KIM5 mutant express lower levels of these messages, whereas those infected with the deletion mutant retain strong expression. Significantly, mRNA for the IL15 receptor alpha chain is not expressed in NK cells from KIM5-infected mice as early as day 2 post infection.

These findings suggest that YopM interferes with innate immunity by depleting NK cells, possibly by affecting the expression of IL15 receptor alpha and IL15 (Kerschen *et al.* 2004).

### Therapies

- Whereas prophylaxis and therapy with ciprofloxacin successfully protects Porton outbred mice infected aerogenically with either GB or CO-92 strains of *Y. pestis* for up to 24 hours after challenge, doxycycline prophylaxis and therapy are ineffective (Russell *et al.* 1998).
- Experiments with adult female Hsd:ND4 mice infected with aerosolized *Y. pestis* revealed that whereas netilmicin, gentamicin, ciprofloxacin, and ofloxacin may be alternatives to streptomycin for treating pneumonic plague in humans, beta-lactam antibiotics are not recommended (Byrne *et al.* 1998).

### Vaccines

- A sub-unit vaccine containing *Y. pestis* V and F1 antigens protects Porton outbred mice against aerosolized *Y. pestis* far better than does a whole cell vaccine. Protection is attributed to the induction of systemic IgG-mediated immunity to the vaccine's F1 and V antigens (Williamson *et al.* 1997).
- Vaccines made with *Yersinia* outer proteins E, K, and N do not protect BALB/c mice against *Yersinia*, indicating that vaccines supplemented with these proteins are not improved (Leary *et al.* 1999).
- An intranasal vaccine consisting of co-encapsulated subunits of F1 and V *Yersinia* antigens protects BALB/c mice better than does the soluble form of the vaccine (Alpar *et al.* 2001).
- Monoclonal antibodies, administered either alone or together against *Yersinia* F1 and V antigens, protect BALB/c mice against bubonic and pneumonic plague up to 48 hours post infection, even without prior vaccination (Hill *et al.* 2003).
- A recombinant fusion vaccine made with *Y. pestis* F1 and V antigens is very effective in protecting immunocompetent hairless Crl:SKH1-*hrBR* mice infected with *Y. pestis* via fleas (Jarrett *et al.* 2004).

## General Description and Pathology

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Smallpox is caused by Orthopoxvirus variola major. The Orthopoxvirus genus is one of eight commonly recognized genera of vertebrate poxviruses. Three more of its members cause disease in humans: *O. vaccinia* (VACV used as the anti-variola vaccine), cowpox virus (CPXV), and monkey pox virus. *O. variola* was eradicated in 1979 (Schriewer *et al.* 2004).

*O. variola* is most effectively spread via the respiratory route. As little as ten plaque-forming units in aerosolized saliva can transmit the infection from person to person (Wehrle *et al.* 1970, in Hassett 2003). Symptoms start to appear between 7 and 17 days after infection. They include high fever, general malaise, head and neck aches, and ulcerative lesions in the mouth. The lesions discharge large quantities of highly infectious virus into the saliva. It is at this time that the disease is most communicable. About two to three days later, the characteristic rash, which is more prominent on the face and extremities, appears. By the second or third week after infection, all lesions are scabbed over, separate from the skin, and leave behind prominent

scars (www.bt.cdc.gov ; Hassett 2003).

In 1971, routine smallpox vaccination with *O. vaccinia* (VACV) was discontinued in the United States. However, it was continued for several more years in other countries. In 1982, it was discontinued for travelers to smallpox-endemic areas. In the United States, the only people who are still vaccinated are Orthopoxvirus researchers, designated emergency first responders, and selected members of the military (Goldsmith *et al.* 2004). The possibility that smallpox may be used in a bioterrorist attack has renewed efforts to develop improved therapies and vaccines. Although a single dose of VACV provides long-term protection, nearly 1 in 1000 vaccinated individuals report complications. About 1 in 20,000 vaccinated children under a year old develop encephalitis, and 30–50% of them die (Brandt *et al.* 2005; Tscharke *et al.* 2005). Additionally, VACV causes complications in immunodeficient individuals (Brandt *et al.* 2005; Goldsmith *et al.* 2004; Smee and Sidwell 2003).

## Smallpox and the Mouse

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Humans are the only natural host of *O. variola*. Attempts to develop animal models of human smallpox have met with limited success (Hassett 2003). The mouse has been the most popular. Although human and mouse immune systems are not identical, the basic principles by which they help protect against various poxviruses are similar (Tscharke *et al.* 2005). Generally, mice are inoculated in the respiratory tract with *O. vaccinia*, cowpox, or ectromelia (“mousepox”), all surrogates for *O. variola*. Ectromelia is the best because it is infectious at very low doses. It is associated with high mortality in the susceptible A, BALB/c, and DBA/2 strains. C57BL/6 mice are resistant (Schriewer *et al.* 2004). Resistance correlates with strong innate and cell-mediated defense mechanisms, including NK cells, the expression of IFN $\alpha$ , IFN $\beta$ , and IFN $\gamma$ , the production of inducible nitric oxide, and, in the case of ectromelia, the rapid activation

and expansion of cytotoxic T lymphocytes that produce high levels of the TH1 cytokines, IL2, IFN $\gamma$ , and TNF $\alpha$  (Hassett 2003). Ectromelia-resistant mice also produce high levels of the TH1-promoting cytokine IL12 during the initial stages of infection (Hassett 2003). Various studies indicate that inappropriate expression of TH2 cytokines such as IL4 can enhance the virulence of poxviruses *in vivo* and imply that reduced cytotoxic T cell responses may limit the ability of unvaccinated hosts to successfully combat primary infections (Hassett 2003).

The mouse has been used extensively to determine the pathogenesis of, immune response to, and efficacy of various drugs and vaccines against *O. variola*. Following are brief descriptions of some of the results of those studies in the last ten years.

## Brief abstracts of selected studies

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### Immunocompromised individuals

- Experiments with hairless mice revealed that certain cidofovir treatment regimens may alleviate complications from smallpox vaccination, particularly in immunocompromised individuals (Smee *et al.* 2004).
- Experiments using *scid* mice revealed that intravenous immunoglobulin products from eight American and two European lots contain active vaccinia antibodies that could provide some protection to immunosuppressed people against accidental exposure to Vaccinia (Goldsmith *et al.* 2004).

### Therapies

- Smee and Sidwell (2003) review 1) the efficacy of selected compounds in fighting non-lethal tail infections, lethal encephalitis, and respiratory infections in mice (including immunosuppressed mice) and 2) the relevance of various animal models for predicting efficacy against orthopox virus infections.
- A 5% topical application of the drug cidofovir most effectively reduces virus titers in skin, lung, kidney, and spleen of immunocompetent hairless (SKH-1) mice inoculated cutaneously with either CPXV or VACV (Quenelle *et al.* 2004).
- Topical treatment of VACV-infected hairless mice with 1%-cidofovir cream reduces the severity of primary lesions, the number of satellite lesions, and the time to death much more effectively than does parenteral cidofovir treatment. Combining both treatments works even better (Smee *et al.* 2004).
- Nude (athymic) mice (NMRI-*nu* or *nu/nu* mice, obtained from Elevage Janvier, Le Genest Saint Isle, France) inoculated in the lumbosacral area with VACV are completely protected against smallpox if treated topically with cidofovir within a day post infection, partially protected when treated topically from day 2 to 5 post infection, and survivors are nearly completely healed when systemically treated two weeks post infection, suggesting that cidofovir may heal disseminated vaccinia lesions in humans experiencing complications from vaccination against smallpox (Neyts *et al.* 2004).

### Vaccines

- Vaccines made with significantly lower doses of attenuated mutant versions of virulent wild-type VACV strains fully protect C57BL/6, BALB/c, and *scid* mice (on the CB17 background) from infections with wild-type VACV strains, suggesting that human vaccines based on mutated wild-type VACV strains would cause fewer complications, such as encephalitis in children, than do VACV vaccine currently being developed (Brandt *et al.* 2005).
- A VACV expression library containing each of 258 predicted open reading frames is used to identify five peptide determinants that account for approximately half of the VACV-specific CD8+ T cell response in C57BL/6 mice (Tschärke *et al.* 2005).
- From 1973 to 1975, a highly attenuated LC16m8 (m8) smallpox vaccine licensed in Japan because of its extremely low neurovirulence was administered to over 100,000 Japanese infants. It induced immunity similar to that of the originating Lister strain, without any serious side effects. However, the m8 virus reverts spontaneously to large plaque forming clones whose virulence equals that of LC16mO, a parental virus strain of m8. By deleting B5R (the gene responsible for the reversion) from m8, a more genetically stable vaccine can be constructed. It is as protective as the U.S.-licensed Dryvax, much superior to modified vaccinia Ankara in a mouse model, and never elicits symptoms in *scid* mice, even when doses are 1000 times greater than those used in the immune protection experiments (Dryvax is lethal to *scid* mice). Thus, this may be a suitable smallpox vaccine (Kidokoro *et al.* 2005).
- Intradermal administration of an HIV-1 vaccine using recombinant modified vaccinia virus Ankara (MVA) as a vector is safe and effective in simian immunodeficiency virus (SIV)-infected rhesus macaques and *Scid* mice. These results indicate that the MVA-vectored HIV-1 vaccine is ready for phase I clinical trials in HIV-1-infected humans, and that similar MVA-vectored vaccines may be efficient against smallpox (Hanke *et al.* 2005).

## *Brief abstracts of selected studies (cont.)*

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- A smallpox vaccine made with recloned modified vaccinia virus Ankara (MVA) safely protects B-cell deficient mice, B2m-deficient mice, BALB/c mice, and BALB *Scid* mice (even at 100 times the dose of vaccinia virus derived from the licensed Dryvax vaccine). In BALB/c mice, it induces a dose-dependent production of virus-specific CD8<sup>+</sup> T cells and antibodies in numbers that equal or exceed those induced by Dryvax. The antibodies induced by MVA and Dryvax are neutralizing and inhibit virus spread in cultured cells. The vaccine also protects these mice against a lethal intranasal challenge with a pathogenic vaccinia virus. In contrast, the vaccine either does not protect or poorly protects CD4<sup>-</sup>, MHC class II<sup>-</sup>, and MHC class I- and II-deficient mice. These results confirm the safety of MVA and demonstrate that the overlapping immune responses protect normal and partially immune-deficient mice (Wyatt *et al.* 2004).
- The antibody and cytotoxic T-cell responses induced in CBySmn.CB17-*Prkdc<sup>scid</sup>*/J and BALBc/J mice vaccinated with a defective and non-replicating vaccinia Lister/Elstree virus are comparable to those induced by the modified vaccinia virus Ankara (MVA). As with the MVA vaccine, the defective vaccine is tolerated at 10,000 times the dose of the wild-type Lister vaccine. Additionally, whereas current nonreplicating vectors are produced mainly in primary chicken cells, this virus can be produced in a permanent safety-tested cell line. The defective vaccine vector is safe *in vitro* and *in vivo*, and it promises to be effective not only for smallpox but for diseases such as cancer, HIV(AIDS), and malaria (Ober *et al.* 2002).

# Tularemia

## General Description and Pathology

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Tularemia, sometimes called “rabbit fever,” is a very infectious and potentially serious illness that occurs naturally in the United States. It is caused by the gram-negative facultative intracellular bacterium *Francisella tularensis* and is found especially in rodents, rabbits, and hares. As few as 10 to 15 bacteria can cause disease, which can be fatal if not treated with proper antibiotics. Depending on the type of exposure, symptoms usually appear in three to five days, sometimes several weeks, and include sudden fever, chills, headaches, diarrhea, muscle aches, joint pain, dry cough, progressive weakness, chest pain, bloody sputum, ulcers on the skin and mouth, swollen and painful lymph glands, swollen and painful eyes, sore throat, breathing difficulties, and pneumonia ([www.bt.cdc.gov](http://www.bt.cdc.gov)).

People contract tularemia in several ways, including through tick, deerfly, and other insect bites, handling infected animal carcasses, ingesting contaminated food and water, and breathing in the bacteria. It is not known to

spread from person to person ([www.bt.cdc.gov](http://www.bt.cdc.gov)).

If used as a weapon, *F. tularensis* would likely be made airborne, and people would become infected by inhaling it. Manufacturing an effective aerosol weapon would require considerable sophistication ([www.bt.cdc.gov](http://www.bt.cdc.gov)).

*F. tularensis* has two predominant subspecies, *tularensis* (commonly referred to as type A) and *holarctica* (commonly called type B). *Tularensis* is found only in North America, is highly virulent, and, if untreated, kills 5 to 10% of its victims (Stenmark *et al.* 2003); if inhaled, it kills 30-60% of its victims (Conlan *et al.* 2005). In contrast, *holarctica* occurs more widely over the Northern hemisphere, is the only subspecies isolated in Europe, is less virulent than type A, and is nonfatal in humans (Stenmark *et al.* 2003).

A new vaccine for tularemia is under review by the Food and Drug Administration and is not currently available in the United States ([www.bt.cdc.gov](http://www.bt.cdc.gov)).

## The LVS Vaccine

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The only clinically useful anti-tularemia vaccine that exists is the Live vaccine strain (LVS) vaccine. It has been shown to immunize humans against type A tularemia. Although it was developed 50 years ago, the genetic nature of its attenuation, its protective antigens, and the immunological basis for its efficacy are unknown. These and other issues have slowed its clinical development and led to calls for its replacement with better-defined vaccines of equal or greater efficacy (Conlan *et al.* 2005). Still, LVS is the gold standard against which new

vaccines must be compared (Chen *et al.* 2003).

The overall impression from epidemiological data and studies in humans, guinea pigs, and monkeys is that LVS protects well against large intradermal infections and small aerosol infections, but not against large aerosol infections of Type A virulent strains (Conlan *et al.* 2005). In humans, monkeys, and guinea pigs, aerosol immunization works much better than does systemic immunization (Eigelsbach *et al.* 1961; Hornick and Eigelsbach 1966).

## Tularemia and the Mouse

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Much of the recent research on the pathogenesis of and immune response to tularemia and the efficacy of the LVS vaccine in the mouse model has been conducted by Conlan in Canada, Elkins in the United States, and their colleagues (see below). Whereas the few early studies used mouse strains that are not readily available today, Conlan's work

focuses on more commonly available strains. Elkins and her colleagues wrote an excellent review of how the mouse has been used in the study of tularemia (Elkins *et al.* 2003). Some of the more significant findings of these and several other researchers are briefly summarized below.

## Differential strain susceptibilities

- Although the lethal dose of LVS (administered intraperitoneally, intravenously, or intranasally) is less than 10 CFUs for strains A/J, BALB/cHsD, C3H/HeNHsD, and SWR/J, it is only 1.5 and 2.7 CFUs for strains C3H/HeJ and C57BL/6J respectively. In contrast, when injected intradermally, mice of all these strains survive infection and an otherwise lethal intraperitoneal re-challenge with as many as  $10^4$  CFUs. Differential susceptibility is due only partly to local dermal site effects. Immunity can be transferred to naïve mice with serum, whole spleen cells, or nonadherent spleen cells from intradermally infected mice (Fortier *et al.* 1991).
- Although LVS-vaccinated BALB/c mice are immune to systemic challenge with virulent Types A and B *F. tularensis*, LVS-vaccinated C57BL/6 mice are only immune to systemic challenge with Type B *F. tularensis*. Neither strain immunized with LVS resists aerosol challenge Type A; only immunized BALB/c mice resist aerosol Type B (Chen *et al.* 2003).
- Median time to death and survival rate of TLR4-defective C3H/HeJ and C57BL/10ScNJ mice infected with low doses of aerosolized type A *F. tularensis* and C3H/HeOuJ controls are comparable. C3H/HeJ mice and C3H/HeOuJ controls have comparable inflammatory responses (Chen *et al.* 2004).
- Of nine common mouse strains (BALB/c, C3H/HeN, CD1, CDF1, 129, CF1, DBA/2, Swiss Webster, and A/J), all but A/J can be immunized by systemic LVS against massive intradermal type A infections but not against low dose aerosolized type A infections. C3H/HeN mice resist such infections the longest (Shen *et al.* 2004).
- Intranasal vaccination with attenuated *F. tularensis* LVS protects BALB/c mice but not C57BL/6J mice against intranasal and subcutaneous *F. tularensis* biovar A (NMFTA1) infection. The protective response requires CD4 and CD8 T cells (Wu *et al.* 2005).
- The median lethal dose (MLD) for either intraperitoneal or intravenous delivery of *F. tularensis* LVS is greater in BALB/c than it is in C57BL/6 mice. The MLD for subcutaneous delivery is comparable in both strains. Whereas LVS-immunized BALB/c mice are fully protected when infected with 1000 times the MLD of either *F. tularensis tularensis* or *F. tularensis holarctica*, immunized C57BL/6 mice are protected only against *F. tularensis holarctica* (Green *et al.* 2005).

## Brief abstracts of selected studies

### Immune responses

- C57BL/6 and A/J mice receiving daily doses of IFNG for three days after being infected with *F. tularensis* LVS resist infection significantly better than do controls (Anthony *et al.* 1989).
- C3H/HeN mice treated with either anti-IFNG or anti-TNFA less than three days after infection are significantly less able to resist infection than are controls, suggesting that T-cell-independent events early in the course of infection suppress the bacteria until a T-cell-dependent response clears them. In contrast, the  $LD_{50}$  for mice treated with anti-IL4 is higher (but not significantly so) than for controls. Additionally, though spleen cells from immune mice normally passively transfer protective immunity to recipient mice in the absence of confounding antibody-mediated immunity, they do not do so if the recipient mice are treated with either anti-IFNG or anti-TNFA at the time of infection (Leiby *et al.* 1992).
- Experiments using BALB/cByJ, C57BL/6J, B6129PF2/J, CD4+ T cell-deficient, *B2m*-deficient, *Tcrb*-deficient, *Tcrd*-deficient, and *Tcrb* + *Tcrd*-deficient mice reveal that either CD4+ or CD8+ T cells appear to be sufficient for resolving a large sub lethal intradermal LVS challenge and for protecting against a maximum secondary lethal LVS challenge (Yee *et al.* 1996; Conlan *et al.* 1994).
- Experiments with B-cell deficient mice reveal that B cells have a previously unappreciated and unexplained role in conferring secondary immunity to *F. tularensis* and other intracellular pathogens (Elkins *et al.* 1999).
- Within 2 to 3 days, treating mice with surprisingly little LVS LPS confers very strong and long-term protection against lethal LVS challenge. Protection does not depend on either the proliferation or secretion of polyclonal immunoglobulin by B cells, nor on the secretion of IL4, IL6, IL12, or IFNG by splenocytes. LPS immunization induces a weak specific anti-LPS IgM response and very

## Brief abstracts of selected studies (cont.)

little IgG; however, infecting mice with LVS bacteria induces vigorous IgM and IgG (particularly IgG2a) anti-LPS antibody responses. Studies using various immunodeficient mouse strains, including LPS-hypo responsive C3H/HeJ mice, B-cell-deficient mice, and *Ifng*-deficient mice, revealed that protective mechanisms do not involve recognition through the *Tlr4<sup>lps-n</sup>* gene product; nonetheless, protection is dependent on B cells and IFNG (Dreisbach *et al.* 2000).

- B cells are not required to generate effective memory T cells: LVS-primed B6.129S2-*Igh-6<sup>tm1Cgn</sup>*/J mice and controls generate comparable quantities of CD4+ and CD8+ T cells and appear to control LVS with IFNG and nitrous oxide. However, they cannot survive secondary LVS infection, presumably dying from a marked neutrophil influx into the spleen very early after infection. If reconstituted with naive B cells before a secondary infection, they survive (Bosio and Elkins 2001).
- A mechanism dependent on the IL12 p40 protein, either alone or in another complex (such as the newly discovered heterodimer IL23), appears to be responsible for clearing LVS infections (Elkins *et al.* 2002).
- Although neutrophils and IFNG are critical for combating early stages of primary systemic tularaemia infection, they appear not to combat early inhaled infections of the lower airways, indicating that it is impossible to predict effective host defense mechanisms against inhalation-initiated tularaemia from current knowledge (Conlan *et al.* 2000a).
- Three T cell subsets in cultures of macrophages from either IFNR-deficient 129S1/SvImJ mice or their controls are equally capable of inhibiting intracellular LVS growth largely by TNF, independent of interferon receptors (Cowley and Elkins 2003).
- Experiments comparing how B-cell-deficient C57BL/10 mice injected with immune serum and those injected with normal serum respond to either the live vaccine strain (LVS) or a clinical isolate of *F. tularensis holarctica* (type B) revealed that specific antibodies help protect mice against *F. tularensis holarctica* (type B) (Stenmarka *et al.* 2003).
- Experiments with BALB/cJ mice revealed that though LPS recognition may not be critical for protection against tularemia, the ability of *F. novicida* LPS to stimulate the production of proinflammatory cytokines (including TNFA) likely contributes to its being more virulent than *F. tularensis*. *F. novicida* LVS and *F. tularensis* disseminate similarly and grow comparably in bone marrow-derived macrophages. However, whereas high doses of *F. novicida* LPS stimulate very modest proliferation of mouse splenocytes *in vitro*, *F. tularensis* LVS LPS does not. Although *F. novicida* LPS-treated macrophages produce IL12 and TNF-alpha, but no detectable IFNG, IL10, or nitric oxide, those treated with *F. tularensis* LVS LPS produce none of these mediators. Both types of purified LPS stimulate early protection against lethal *F. tularensis* LVS, but not against lethal *F. novicida* LVS (Kieffer *et al.* 2003).
- Mice given *F. tularensis*-specific antibodies have more TNFA, IL12, and skin neutrophils after being infected cutaneously with LVS than do controls (Chen *et al.* 2004a).
- Within six to eight days, B6.129S7-*Ifng<sup>tm1Ts</sup>*/J, B6.129S-*Tnfrsf1a<sup>tm1Imx</sup> Tnfrsf1b<sup>tm1Imx</sup>*/J, B6.129P2-*Nos2<sup>tm1Lau</sup>*/J, B6.129S2-*Igh-6<sup>tm1Cgn</sup>*/J, and control mice all succumb to low doses of either aerosol or intradermal challenges with virulent type B *F. tularensis*. *Ifng*- and *Igh-6*-deficient mice consistently harbor up to one log(10) more bacteria in their lungs, spleens, and livers than do controls at day five post-aerosol exposure. Compared to other strains examined, *Ifng*-deficient mice have only mild liver damage. Even mice with broad immunodeficiency (*Scid* mice on a BALBc background, neutropenic, splenectomized or thymectomized mice and mice treated with corticosteroid) are no more susceptible to aerosol-initiated infection with either virulent type B or type A *F. tularensis* than are controls. Thus, unlike LVS, normal type A and type B *F. tularensis* strains are so extremely virulent that even immunocompetent mice are virtually defenseless to low dose aerosol and intradermal challenges (Chen *et al.* 2004b).
- *F. tularensis* immunity in BALB/c and C3H/HeN mice depends on IFNG, conventional  $\alpha/\beta$ T cells, and on limiting the dissemination of the pathogen from the lungs to other internal organs (Conlan *et al.* 2005).
- At least 36 different *F. tularensis* proteins, 27 of them previously undescribed, strongly react with sera from experimentally infected C3H/HeN and C3H/HeJ mice. The proteins include several putative virulence markers of intracellular pathogens, such as nucleoside diphosphate kinase, isocitrate dehydrogenase, RNA-binding protein Hfq, and molecular chaperone ClpB. Many of the proteins are also recognized by sera collected from humans with

## Brief abstracts of selected studies (cont.)

tularemia. Although the serum immunoreactivity to *F. tularensis* in the two mouse strains varies throughout the course of infection, the *F. tularensis* antibodies produced by the two strains are comparable (Havlasova *et al.* 2005).

- C.129S7(B6)-*Ifng*<sup>tm1Ts</sup>/J, C.129S1(B6)-*Il12a*<sup>tm1Jm</sup>/J (p40-deficient), and C.129S1-*Il12b*<sup>tm1Jm</sup>/J (p35-deficient) mice succumb to intranasal LVS doses that are sub lethal to wild-type controls. Treating BALB/c and C57BL/6 mice with exogenous IL12 twenty-four hours before a lethal intranasal LVS dose significantly decreases their lung, liver, and spleen bacterial loads, and they survive. C.129S7(B6)-*Ifng*<sup>tm1Ts</sup>/J mice treated similarly die. C57BL/6J-*Lys*<sup>tg-1</sup>/J (NK cell-deficient) mice also resist LVS via IL12, but B6.129S2-Cd8atm1Mak/J mice do not. Thus, IFNG and IL12 are strictly required for LVS protection, and intranasally delivered IL12 can prevent respiratory tularemia through a mechanism that is at least partially dependent on IFNG and CD8 T cells (Duckett *et al.* 2005).
- Although LVS replicates within mouse and human macrophages, it induces different immune responses in each. In response to either live or killed LVS, human monocyte-derived macrophages release large amounts of CXC chemokine ligand 8 (CXCL8) and CC chemokine ligand 2; in response to live LVS, they release IL1B; in response to various bacterial preparations, human monocytes secrete CXCL8, IL1B, and TNFA. In contrast, LVS preparations of all kinds induce little to no proinflammatory cytokines and chemokines in

mouse bone marrow-derived macrophages. The greater proinflammatory response of human leukocytes to LVS may be one of the reasons why LVS is not virulent in humans but is lethal in mice (Bolger *et al.* 2005).

### Vaccines

- A vaccine consisting of the O-polysaccharide of *F. tularensis* LPS chemically conjugated to bovine serum albumin completely protects BALB/c mice against some types of highly virulent type B *F. tularensis* infections but does not protect them against virulent type A *F. tularensis* infections (Conlan and Shen *et al.* 2002b).
- Whereas either aerogenic or intradermal LVS vaccines immunize BALB/c and C3H/HeN mice against massive intradermal type A infections, only aerogenic vaccines immunize them against aerosolyzed type A infections (Conlan *et al.* 2005).
- A rare population of CD4(-)CD8(-)CD3(+)alphabeta(+)gammadelta(-)NK1.1(-) T cells functions very much like memory T cells, including the well-known CD4(+) and CD8(+) T cells. They potently and specifically inhibit the growth of either *Mycobacterium tuberculosis* or *Francisella tularensis* LVS in macrophages *in vitro*, help C57BL/6J mice survive these infections, and adoptively transfer immunity to *F. tularensis* LVS. Furthermore, they expand in the spleens of infected mice and acquire a memory cell phenotype. They may be important for successful vaccination (Cowley *et al.* 2005).

# JAX<sup>®</sup> Mice Models for Immunology Research

The Jackson Laboratory offers hundreds of inbred, mutant, and other JAX<sup>®</sup> Mice inbred, mutant, and other strains suitable for infectious disease research.

- Inbred strains may be used to develop new models of disease (such as was done for Ebola).
- JAX<sup>®</sup> Mice Macroarrays (panels of JAX<sup>®</sup> Mice - see below) may be used to identify susceptible and resistant strains, determine the genetic bases of disease susceptibility, characterize the pathogenesis and immune responses to infectious disease, and develop and test therapies and vaccines.
- Mutant strains may be used to verify and better characterize the genetic bases of immune responses to infectious agents.

A PDF of Jackson Laboratory immunology research models is available at the Web site [www.jax.org/jaxmice/models/immunology](http://www.jax.org/jaxmice/models/immunology). The models are subdivided into the following categories (many are found in more than one category):

- Autoimmunity
- CD Antigens, Antigen Receptors, and Histocompatibility Markers
- Growth Factors/Receptors/Cytokines
- Immunodeficiency
- Immunodeficiency Associated with Other Defects
- Inflammation
- Intracellular Signaling Molecules
- Lymphoid Tissue Defects
- Mechanisms of HIV Infection
- Rearranged Antigen-Specific T Cell Receptor Transgenes
- T Cell Receptor Signaling Defects
- Vaccine Development

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In the following pages, we describe a few of the models that have been used to research the infectious diseases discussed in the first section of this manual and that could be used for further research.

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## B-cell deficient mice

*Igh-6*

**B6.129S2-*Igh-6*<sup>tm1Cgn</sup>/J (002288)**

These *Igh-6*<sup>tm1Cgn</sup> homozygotes are viable and fertile. They lack mature B-cells and do not express membrane-bound IgM. *Igh-6*-deficient mice have been used in many studies to understand the role of B cells in the immune system. Some may produce some B-cells using a C gene other than *Igmu*.

LVS-primed B6.129S2-*Igh-6*<sup>tm1Cgn</sup>/J mice and controls generate comparable quantities of CD4+ and CD8+ T cells and appear to control Tularemia live vaccine strain (LVS) with IFNG and nitrous oxide. However, they cannot survive secondary LVS infection, presumably dying from a marked neutrophil influx into the spleen very early after infection. If reconstituted with naive B cells before a secondary infection, they survive (Bosio and Elkins 2001; Chen *et al.* 2004).

*Ifng*- and *Igh-6*-deficient mice all die within 6 to 8 days of either aerosol or intradermal challenges with virulent type B *F. tularensis*.

They consistently harbor up to one log(10) more bacteria in their lungs, spleens, and livers than do controls at day five after aerosol exposure. Unlike LVS, normal type A and type B *F. tularensis* strains are so extremely virulent that even immunocompetent mice are found to be virtually defenseless against low dose aerosol and intradermal challenges (Chen *et al.* 2004).

Th1 T-cell responses of *Igh-6*-deficient mice on a C57BL/6 background are impaired early in and throughout a primary infection with *Salmonella enterica serovar Typhimurium*. These mice produce less Th1 IFNG than do controls and soon switch over to a Th2 response. *In vitro*, B cells upregulate co stimulatory molecules when stimulated with *S. enterica serovar Typhimurium* and present Salmonella antigens to Salmonella-specific CD4(+) T cells. The critical role B cells play in Th1 responses may be due to their function as APCs (Ugrinovic *et al.* 2003).

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## Cytokine-deficient mice

*Chemokine- and chemokine receptor-deficient mice*

***Ccr2***

**B6.129S4-*Ccr2*<sup>tm1lfcr</sup>/J (004999)**

B6.129S4-*Ccr2*<sup>tm1lfcr</sup>/J homozygotes are viable, fertile, and appear and behave normally, but they have defective type 1 immune responses. They produce reduced amounts of IFN $\alpha$  in response to induced lung granulomas, recruit reduced numbers of peritoneal macrophages when challenged with thioglycollate, and their macrophages fail to migrate in response to monocyte chemoattractant protein-1 (MCP-1) (Boring *et al.* 1997).

Experiments with *Ccr2*-deficient mice on a BALB/c background and controls reveal that alveolar monocyte recruitment (but not LPS-induced neutrophil migration to the lungs) is dependent on CCR2. However, CCR2-bearing

blood monocytes dramatically increase alveolar neutrophil accumulation. These results reveal that leukocyte efflux in response to lung inflammation is mediated by a previously unrecognized cooperation between monocytes and neutrophils (Maus *et al.* 2003).

Experiments using *Ccr2*-, *Ccr5*-, and *MIP-1a*-deficient mice on an outbred C57BL/6J x 129/Ola background and C57BL/6J x 129/Ola controls (which are resistant to *Leishmania major*) infected with *L. major* revealed that CCR2 is an important determinant not only of dendritic cell migration and localization but of the development of protective cell-mediated immune responses to *L. major* (Sato *et al.* 2000).

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## Cytokine-deficient mice

### Interferon gamma-deficient mice

**Ifng**                      **B6.129S7-Ifng<sup>tm1Ts</sup>/J (002287)**

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Interferon gamma (IFNG) is a pleiotrophic cytokine that modulates and is essential for the proper functioning of a variety of immune cells. B6.129S7-*Ifng*<sup>tm1Ts</sup>/J homozygotes are viable, fertile, and appear normal in the absence of pathogens. However, in their presence, their macrophages produce relatively few antimicrobial products and MHC II antigens. In response to mitogens and alloantigens, their splenocytes proliferate uncontrollably. Additionally, the activity of their resting NK cells is reduced.

Whereas B6.129S7-*Ifng*<sup>tm1Ts</sup>/J mice infected with a slowly invasive strain of lymphocytic choriomeningitis virus (LCMV Armstrong) become chronically ill, controls rapidly clear the infection. *Ifng*-deficient mice generate at least as many cytotoxic T-lymphocyte (CTL) effectors as do controls, indicating that, in the absence of IFNG, their CTLs cannot clear the infection. Rather, they seem to be kept permanently activated by the continuous presence of live virus (Bartholdy *et al.* 2000).

Leishmania-infected mice vaccinated with developmentally regulated *Leishmania amastigote*

antigen P-8 are initially protected by CD8(+) T cells secreting IFNG and expressing perforin. Eventually, the activated CD4(+) T cells primarily produce IFNG. Protection correlates with the ratio of total IFNG-producing cells (CD4(+) and CD8(+) T cells) to macrophages at the site of infection. Vaccinating B6.129S7-*Ifng*<sup>tm1Ts</sup>/J, B6.129P2-*B2m*<sup>tm1Unc</sup>/J, and C57BL/6-*Prf1*<sup>tm1Sdz</sup>/J mice with the developmentally regulated *Leishmania amastigote* antigen P-8 does not protect them against Leishmania, indicating that CD8(+) T cell effector mechanisms (MHC1 APC presentation, perforin, IFNG) are required to protect mice against *L. amazonensis* (Colmenares *et al.* 2003).

B6.129S7-*Ifng*<sup>tm1Ts</sup>/J, B6.129S2-*Igh-6*<sup>tm1Cgn</sup>/J, and control mice all succumb to low doses of either aerosol or intradermal challenges with virulent type B *F. tularensis* within 6 to 8 days. They consistently harbor up to one log (10) more bacteria in their lungs, spleens, and livers than do controls at day 5 post aerosol exposure. Compared to other strains examined, *Ifng*-deficient mice have only mild liver damage (Chen *et al.* 2004).

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## Cytokine-deficient mice

### Interleukin-deficient mice

**IL4**

**B6.129P2-*Il4*<sup>tm1Cgn</sup>/J (002253)**

Interleukin-4 (*Il4*) deficient mice have frequently been used to determine the role of IL4 in the immune response. *In vitro*, IL4 promotes the growth and differentiation of many hematopoietic cells. In particular, it directs the immunoglobulin (Ig) class switch to IgG1 and IgE. B6.129P2-*Il4*<sup>tm1Cgn</sup>/J homozygotes develop normal T and B cell but have severely reduced serum IgG1 and IgE levels. They completely lack the typical IgG1 dominance of a T cell-dependent immune response, and they produce no detectable IgE in response to a nematode infection. Studies of these mice reveal that only some of the *in vitro* properties of IL4 are critical for the physiology of the immune system *in vivo* (Kuhn *et al.* 1991).

After being tick-bite infected with *Borrelia burgdorferi*, BALB/c-*Il4*<sup>tm2Nnt</sup>/J mice produce significantly greater titers of spirochete-specific IgG2a than do controls, which produce

significantly more spirochete-specific IgG1. *In vitro*, *Il4*-deficient and control splenocytes proliferate similarly post-infection. However, at day 30, antigen-stimulated *Il4*-deficient splenocytes produce significantly more IFNG than do those from controls, suggesting that Th1-influences responses predominate in *Il4*-deficient mice. Moreover, inflamed hearts from *Il4*-deficient mice have higher levels of IFNG and TNFA transcripts than do those of controls. Antigen-stimulated splenocytes from both kinds of mice produce significant amounts of IL10, but those from *Il4*-deficient mice produce either no or little IL4. Although *Borrelia*-infected *Il4*-deficient mice develop a more severe carditis on day 30, it is resolved by day 50, as it is in controls, indicating that although IL4 may help limit the severity of Lyme carditis, its absence does not preclude resolution.

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## Cytokine-deficient mice

*Interleukin-deficient mice (cont.)*

**Il6**                      **B6.129S2-Il6<sup>tm1Kopf</sup>/J (002650)**

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Interleukin-6 (IL6) is a multifunctional cytokine that regulates various aspects of the immune response, acute-phase reaction, and hematopoiesis. *In vitro*, its activities overlap with those of leukemia inhibitory factor, oncostatin M, ciliary neurotrophic factor, and IL11: these molecules and Il6 have specific binding factors that interact with the gp130 signal-transducing receptor. B6.129S2-Il6<sup>tm1Kopf</sup>/J homozygotes develop normally but cannot efficiently control infections with either vaccinia virus or *Listeria monocytogenes*, a facultative intracellular bacterium. Their T-cell-dependent antibody responses against vesicular stomatitis virus are impaired. Whereas their inflammatory acute-phase responses are only moderately affected after being challenged with LPS, they are

severely compromised after tissue damage or infection. Studies of these mice have revealed that injury- and infection-induced production of IL6 coordinates the activities of liver cells, macrophages, and lymphocytes (Kopf *et al.* 1994).

*Yersinia enterocolitica*, a gram-negative enteric pathogen responsible for a number of gastrointestinal disorders, is considerably more virulent in B6.129S2-Il6<sup>tm1Kopf</sup>/J mice and colonizes systemic tissues more rapidly and to a higher degree than it does in controls. Il6-deficient mice have a more robust T(H)1 T-cell response than do controls. Their hyperinflammatory pathologies appear to be due to the misregulation of TNFA, monocyte chemoattractant protein 1, IL10, TGF beta1, and IFNG (Dube *et al.* 2004).

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## Cytokine-deficient mice

*Interleukin-deficient mice (cont.)*

***Il12a***                      **B6.129S1-*Il12a*<sup>tm1Jm</sup>/J (002692)**  
***Il12b***                      **B6.129S1-*Il12b*<sup>tm1Jm</sup>/J (002693)**

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Interleukin-12 (IL12) regulates T and NK cells and promotes Th1 responses. B6.129S1-*Il12b*<sup>tm1Jm</sup>/J homozygotes mount severely restricted Th1 responses to *in vivo* endotoxins (produce very little IFNG), produce normal amounts of IL2 and IL10, have enhanced Th2 responses (secrete large amounts of IL4), have normal allogeneic CTL responses, and have delayed type hypersensitivity (DTH) responses. Thus, IL12 plays an essential role in regulating IFNG production and in facilitating normal DTH responses but not in other phenomena associated with Th1 responses and cell-mediated immunity (Magram *et al.* 1996). Experiments using B6.129S1-*Il12a*<sup>tm1Jm</sup>/J and B6.129S1-*Il12b*<sup>tm1Jm</sup>/J mice revealed that both subunits of IL12, p40 and p35, are required for continued resistance to *Leishmania major*. Experiments using mice deficient both for *Il4* and *Il12* revealed that, even in the absence of an

*Il4*-induced Th2 response, IL12 is required to maintain resistance. Thus, rather than modulating Th2 responses or optimizing the production of IFNG, IL12 may maintain cell-mediated immunity by preventing the loss of Th1 cells during an infection (Park *et al.* 2002).

IL12 interacts with IFN $\alpha$  and IL15 to activate NK cell responses to viral infections. Whereas IL12/STAT4 is critical for NK cells to express IFNG, IFN- $\alpha$ /STAT1 is needed to induce cytotoxicity. To proliferate and survive, NK cells require IFN- $\alpha$ /STAT1 induction of IL-15 (but do not need STAT4) (Nguyen *et al.* 2002).

A mechanism dependent on the IL12 p40 protein, either alone or in another complex (such as the newly discovered heterodimer IL23), appears to be responsible for clearing LVS infections (Elkins *et al.* 2002).

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## Cytokine-deficient mice

### Interleukin-deficient mice (cont.)

#### ***Il18***

#### **B6.129P2-*Il18*<sup>tm1Aki</sup>/J (004130)**

IL-18 is secreted from activated macrophages and induces IFNG production. B6.129P2-*Il18*<sup>tm1Aki</sup>/J homozygotes appear and behave normally. When infected with *Propionibacterium acnes*, they produce markedly reduced amounts of IFNG, despite normal IL12 induction, and their NK cell activities are significantly impaired. After they are infected with either *P. acnes* or *Mycobacterium bovis* (*bacillus Calmette-Guerin* [BCG]), their Th1 cell responses are significantly reduced. In contrast, Th1 responses of *Il12*-deficient mice are induced after BCG infection. However, NK activity and Th1 responses of mice deficient both in *Il18* and *Il12* are even more seriously impaired than are those of *Il18*-deficient mice, demonstrating the important role of both IL18 and IL12 in NK activity and Th1 responses (Takeda *et al.* 1998).

In the first few days after being infected intranasally with the mouse-adapted strain of human influenza A/PR/8/34 (H1N1) virus, *Il18*-deficient C57BL/6 mice have a higher mortality rate than do controls. Pathogenic changes include pronounced virus growth, massive infiltration

of inflammatory cells, and elevated nitric oxide production. IFNG levels are significantly lower, but IL12 levels are slightly higher than they are in controls. NK cell-mediated cytotoxicity is poorly activated. Local immune responses, such as specific cytotoxic T lymphocyte and antibody production, are induced as well as they are in the controls. Thus, IL18 helps control early pulmonary influenza by activating innate immune mechanisms such as IFN and NK cells (Liu *et al.* 2004).

The role of IL18 in resistance to *L. major* is determined by host genetic background. *Il18*-deficient 129 x CD1 mice are more susceptible to *Leishmania major* than are controls; *Il18*-deficient C57BL/6 mice are as resistant as controls; *Il18*-deficient BALB/c mice are more resistant than are the highly susceptible BALB/c controls; *Il18*-deficient DBA/1 mice are markedly more susceptible than are the moderately resistant DBA/1 controls. Whereas *Il18*-deficient BALB/c mice produce less IFNG and IL4, *Il18*-deficient DBA/1 mice produce more IFNG and IL4 than do controls (Wei *et al.* 2004).

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## Cytokine-deficient mice

### *Nos2*-deficient mice

***Nos2***                      **B6.129P2-*Nos2*<sup>tm1Lau</sup>/J (002609)**

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These *Nos2*<sup>tm1Lau</sup> homozygotes are viable, fertile, and appear normal. Unlike NOS1 and NOS3, NOS2 is synthesized *de novo* in response to a variety of inflammatory stimuli. Induction of *Nos2* results in the production of large amounts of nitric oxide (NO) over prolonged periods of time. Excessive NO production is beneficial through its antitumor and antimicrobial activities. It is also thought to damage tissues and contribute to pathology in a variety of inflammatory conditions, including rheumatoid arthritis, inflammatory bowel disease, cardiac allograft rejection, hepatotoxicity, myocardial ischemia-reperfusion, and septic shock. NO helps regulate blood pressure and hemodynamics. In response to LPS, B6.129P2-*Nos2*<sup>tm1Lau</sup>/J homozygotes produce virtually no serum NO, but they are susceptible to LPS-induced death. They respond aberrantly to *M. bovis* (BCG), systemic *E. coli* infection, *M. tuberculosis*, and *M. pulmonis*. Their fibroblasts have impaired wound healing properties (Laubach *et al.* 1995).

Whereas all controls infected with *Mycobacterium bovis Bacillus Calmette Guerin* (BCG) survive, all infected *Nos2*-deficient mice die within 8 to 12 weeks. They develop large granulomas consisting of macrophages and activated T cells and gaseous necrotic lesions in the spleen. The macrophages have reduced acid phosphatase activities, suggesting that NO is required to activate macrophages. The absence of NOS2 affects the production of Th1 cytokines, except IL18. *Nos2*-deficient mice produce more serum IL12p40, less IFNG, and more TNF than do controls throughout the infection period. TNFR1 and TNFR2 shedding is altered. Perhaps TNF is upregulated to compensate for the lack of NOS2. The late neutralization of TNF by soluble TNFRs increases disease severity and accelerates death in *Nos2*-deficient mice but has no effect in controls. In effect, *Nos2*-deficient mice eventually succumb to *M. bovis* BCG because they overproduce inflammatory cytokines (Garcia *et al.* 2000).

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## Cytokine-deficient mice

### *Tumor necrosis factor-deficient mice*

***Tnf***                      **B6;129S6-*Tnf*<sup>tm1Gkl</sup>/J (003008)**

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B6;129S6-*Tnf*<sup>tm1Gkl</sup>/J homozygotes are viable, fertile, appear and behave normally, and develop normal lymph nodes and Peyer's patches. They readily succumb to *Listeria monocytogenes* infections and show reduced contact hypersensitivity responses. Although they are resistant to the systemic toxicity of LPS upon D-galactosamine sensitization, they are sensitive to high doses of LPS alone. They completely lack splenic primary B cell follicles and cannot form organized follicular dendritic cell networks and germinal centers. Although they are capable of Ig class-switching, their humoral immune responses against either thymus-dependent or thymus-independent antigens are impaired. Transgenic expression of either human or murine TNFA reconstitutes these defects, indicating that TNFA regulates the development and organization of splenic follicular architecture and the maturation of the humoral immune response (Pasparakis *et al.* 1996).

Failure of *Tnf*-deficient mice to clear an *E. coli* infection is associated with decreased systemic concentrations of chemokine macrophage

inflammatory protein-2, reduced pulmonary neutrophil recruitment, and depressed expression of neutrophil CD11b and CD16/CD32 (Lee *et al.* 2003).

C57BL/6 mice are naturally resistant to *Leishmania major*. However, *Tnf*-deficient C57BL/6 mice locally infected with *L. major* rapidly succumb to progressive visceral leishmaniasis, even when the parasite inoculum is as low as 3000 promastigotes. Although they mount an *L. major*-specific IFNG response and express IL12, their immune reaction is delayed. When stimulated by IFNG *in vitro*, *Tnf*-deficient inflammatory macrophages release 10 times less NO than do control cells. However, in the presence of a costimulus, either *L. major* or LPS, *Tnf*-deficient and control macrophages produce comparable amounts of NO. Although inducible NO synthase protein is readily detectable in the skin lesions and draining lymph nodes of *Tnf*-deficient mice and controls, its expression is more diffuse and less focal in the *Tnf*-deficient mice (Wilhelm *et al.* 2001).

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## Cytokine-deficient mice

*Tumor necrosis factor-deficient mice (cont.)*

***Tnfrsf1a Tnfrsf1b* B6;129S-*Tnfrsf1a*<sup>tm1lmx</sup> *Tnfrsf1b*<sup>tm1lmx</sup>/J (003243)**

Mice homozygous for both *Tnfrsf1a*<sup>tm1lmx</sup> and *Tnfrsf1b*<sup>tm1lmx</sup> (p55 and p75 deficient) are viable and fertile. They fail to bind TNF. Thymus, spleen, and other lymphoid tissue are normal, indicating that TNF is not required for normal development of these organs. Acute responses to LPS are unaltered.

CD4(+)-depleted B6;129S-*Tnfrsf1a*<sup>tm1lmx</sup> *Tnfrsf1b*<sup>tm1lmx</sup>/J mice infected with *Pneumocystis pneumonia* (PcP) have significantly less pulmonary RANTES, monocyte chemoattractant

protein 1, macrophage-inflammatory protein 2, cytokine-induced neutrophil chemoattractant responses, inflammatory cell recruitment to the alveoli, and histological evidence of PcP-related alveolitis than do controls, indicating that TNFR signaling is required for maximal CD8(+) T cell-dependent pulmonary inflammation and lung injury during PcP. The data also demonstrate that CD8(+) T cells can use TNFR signaling pathways to respond to an extracellular fungal pathogen (Wright *et al.* 2004).

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## Hemolytic complement-deficient mice

Hemolytic complement (Hc), previously called component 5 (C'5), is a pivotal zymogen of the complement cascade. JAX<sup>®</sup> Mice strains A/HeJ (000645), A/J (000646), AKR/J (000648), B10.D2-*Hc*<sup>0</sup> *H2<sup>d</sup>* *H2-T18<sup>c</sup>*/oSnJ (000461), CE/J (000657), DBA/2J (000671), NZB/BINJ, (000684),

RF/J (000682), and SWR/J (000689) have a two-base pair TA deletion in a 5'exon of the *Hc* gene, resulting in a premature termination codon, aberrant *Hc* transcripts, and either shortened or no gene products. These mice all have immunologic deficiencies.

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## T cell-defective mice

### Cd4

### B6.129S2-*Cd4<sup>tm1Mak</sup>/J* (002663)

B6.129S2-*Cd4<sup>tm1Mak</sup>/J* homozygotes do not express CD4 on their cell surfaces. They develop normal CD8+ T cells and myeloid components, indicating that expression of CD4 on progenitor cells and CD4+ CD8+ (double positive) thymocytes is not obligatory. Although their cytotoxic T-cell activity against viruses is normal, their helper T cell activity against these viruses is markedly reduced. Hence, they may be useful for researching the role of CD4+ helper T cells in immune disorders (Rahemtulla *et al.* 1991).

Experiments with CD4-deficient mice on a BALBc background indicate that either CD4+ cells or cells activated by them may reduce disease resistance after acute cold/restraint stress (ACRS) (Cao *et al.* 2003).

CD4-deficient mice on a C57BL background remain healthy for only about three months after their lungs are infected with the murine gamma-herpesvirus 68 (gammaHV68). Although vaccinating such mice with an HV68-expressing recombinant Vacc-p56 virus greatly increases

their p56-specific CD8(+) T cells, it does not increase their lifespan: they have fewer CD44 glycoproteins on those p56-specific CD8(+) T cells, lower level of CTL activity, and fewer IFNG(+)/TNFA(+) T cells (detected after *in vitro* stimulation with the p56 peptide). To evaluate the efficacy of such vaccines for diseases like HIV(AIDS), the molecular mechanisms that result from CD4-deficiency should be evaluated (Liu *et al.* 2002).

Experiments using CD8 T-cell-deficient mice (B6.129P2-*Tcrb<sup>tm1Mom</sup>/J*), B cell-deficient mice (B6.129S2-*Igh-6<sup>tm1Cgn</sup>/J*), and CD4 T cell-deficient mice (B6.129S2-*Cd4<sup>tm1Mak</sup>/J*) revealed that whereas CD8 cells help protect against acute Ebola infection, CD4 cells and antibodies are required for long-term protection. Under certain immunodeficiency conditions, the virus can persist, and loss of primed CD4 T cells accelerates the course of persistent infections (Gupta *et al.* 2004).

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## T cell-defective mice *(cont.)*

### Cd8

#### B6.129S2-Cd8a<sup>tm1Mak</sup>/J (002665)

B6.129S2-Cd8a<sup>tm1Mak</sup>/J homozygotes have no functional cytotoxic T-cells: CD8 is not expressed on their cell surfaces. They seem to have normal CD4+ T lymphocyte populations. Although their cytotoxic T cell responses against alloantigens and viral antigens are dramatically decreased, their proliferative responses against alloantigens and the help they give to B lymphocytes *in vivo* are normal. This suggests that whereas CD8 is necessary for the maturation and positive selection of class I MHC restricted cytotoxic T cells, it is not necessary for the development of intermediate thymocyte populations (CD8+CD4-TcR- or CD4+CD8+TcRlow) into functional class II MHC restricted helper T cells (Fung-Leung *et al.* 1991).

Infesting B6.129S2-Cd8a<sup>tm1Mak</sup>/J, B6.129P2-B2m<sup>tm1Unc</sup>/J, B6.129S2-Cd4<sup>tm1Mak</sup>/J, B6.129P2-

Tcrd<sup>tm1Mom</sup>/J, B6.129S1-Il12b<sup>tm1m</sup>/J, MHC class II-deficient B6.169-Ab<sup>tm1</sup> N5-M, B6.169-Ab<sup>tm1</sup> N6-W mice, and controls with Armstrong or WE strains of lymphocytic choriomeningitis virus (LCMV) induces an unexpectedly early day 4 IFNG response (detectable in serum samples and spleen and liver homogenates). Although this response does not require IL12, NK cells, TCR-gammadelta T cells, MHC class II, or CD4 T cells, it is MHC class I/CD8-dependent. Peak response does require specific Ag recognition. The IFNG response is associated with IL18 and IFN- $\alpha$  expression. Thus, viral infections induce a dramatic CD8 T cell response that depends on endogenous innate cytokines, which may also act as a mechanism to precisely limit T cell functions to times of need (Pien *et al.* 2002).

### References

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### Cd14

#### B6.129S-Cd14<sup>tm1Frm</sup>/J (003726)

B6.129S-Cd14<sup>tm1Frm</sup>/J homozygotes are viable and fertile. However, their macrophages do not produce cytokines in response to LPS. This unresponsiveness is associated with impaired activation of both the NF- $\kappa$ B and the c-Jun N-terminal mitogen-activated protein kinase (MAPK) pathways. On the other hand, their macrophages do produce cytokines in response to whole bacterial particles: TNF response to concentrations of 10 or more heat-killed

*Escherichia coli* per cell are comparable to those of wild-type macrophages. This CD14-independent cytokine production is substantially reduced by neutrophil inhibitory factor (NIF, a competitive inhibitor of the  $\beta$ 2 integrin CD11B) and by the inhibition of phagocytosis by cytochalasins. This model may be valuable for dissecting the complex mechanisms macrophages use to combat bacterial infections.

### References

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## T cell-defective mice *(cont.)*

### ***B2m***

#### **B6.129P2-*B2m*<sup>tm1Unc</sup>/J (002087)**

B6.129P2-*B2m*<sup>tm1Unc</sup>/J homozygotes appear normal but lack beta 2 microglobulin. Therefore, they express little if any MHC class I proteins, which display viral and self antigens to potentially responsive cells and are important for the maturation of T cells, and they are grossly

deficient in CD4- CD8+ T cells, which normally mediate cytotoxic T cell function. These mice are useful for researching how the interactions among *B2m* and MHC class I molecules regulate T cell development.

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### ***Fcer1g***

#### **B6;129P2-*Fcer1g*<sup>tm1Rav</sup>/J (002847)**

B6;129P2-*Fcer1g*<sup>tm1Rav</sup>/J homozygotes are viable and fertile but lack the gamma subunit of immunoglobulin Fc receptors (*Fcer1g*). *Fcer1g* is an essential component of the high-affinity receptor for IgG (Fc gamma RIII) and is associated both with the high-affinity receptor for IgG (Fc gamma RI) and the T cell receptor-CD3 complex. Without it, those receptors cannot assemble. Although *Fcer1g* homozygotes develop

normal T cells, they are unable to phagocytose antibody-coated particles (despite normal binding), have defective NK cell-mediated antibody-dependent cytotoxicity and mast cell-mediated allergic responses, and attenuated inflammatory responses to immune complexes. These mice could be used to evaluate the role of Fc receptors in humoral and cellular immune responses.

### **References**

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## T cell-defective mice *(cont.)*

### Prf1

### C57BL/6-Prf1<sup>tm1Sdz</sup>/J (002407)

C57BL/6-Prf1<sup>tm1Sdz</sup>/J mice are viable and fertile. Though they possess normal numbers of CD8+ T cells and NK cells, those cells are unable to lyse either virus-infected or allogeneic fibroblasts *in vitro*. These mice also fail to clear lymphocytic choriomeningitis virus, and they inefficiently eliminate fibrosarcoma tumor cells. Thus, perforin is a key effector molecule for T-cell- and natural killer-cell-mediated cytotoxicity (Kagi *et al.* 1994).

C57BL/6-Prf1<sup>tm1Sdz</sup>/J mice infected with influenza virus have a higher mortality rate, more viral growth, and more prolonged viral shedding than do controls. *In vitro*, their infected lung parenchyma have neither NK cell nor virus-specific CTL activities; however, they produce normally functioning antibodies. Apoptosis of virally infected lung cells is delayed and may explain why CD4, CD8, and CD19 positive

cells infiltrate and remain in the lungs for an abnormally long time. IFNG is the most abundant cytokine produced at the site of infection. Thus, perforin seems to play a crucial role in defending against influenza virus, especially in early stage infections, by inducing apoptosis of virus-infected cells (Liu *et al.* 2003).

Whereas *Fas*-deficient mice (B6.MRL-*Fas*<sup>del</sup>/J), 90% of *Ifng*-deficient mice (B6.129S7-*Ifng*<sup>tm1Ts</sup>/J), and all C57BL/6J controls survive sub-cutaneous infections with mouse-adapted Ebola, perforin-deficient mice (C57BL/6-Prf1<sup>tm1Sdz</sup>/J) all die. They fail to clear the infections even though they develop normal levels of neutralizing anti-Ebola antibodies, 5 to 10 times more IFNG, and 2 to 4 times more Ebola-specific CD8 cells than do controls. Thus, clearing Ebola is perforin- rather than IFNG-dependent (Gupta *et al.* 2005).

## References

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## T-cell receptor-deficient mice

*Tcrb*

**B6.129P2-*Tcrb*<sup>tm1Mom</sup>/J (002118)**

B6.129P2-*Tcrb*<sup>tm1Mom</sup>/J homozygotes are viable and fertile. *Tcrb* gene rearrangement or expression is critical for the differentiation of CD4-CD8-thymocytes into CD4+CD8+ thymocytes, and for the expansion of the pool of CD4+CD8+ cells. The number of thymus cells in B6.129P2-*Tcrb*<sup>tm1Mom</sup>/J mice is approximately 8% of that found in controls, and the number of their CD4+CD8+ cells is approximately 6% of that found in controls. The proportion of CD4-CD8- (IL2 receptor positive) cells increases to about 50% of the total cell number. Differentiation of alpha beta thymocytes is blocked earlier than it is in the *Tcr<sup>α</sup>tm1Mom* strain; differentiation of gamma delta thymocytes is normal. These mutants may develop inflammatory bowel disease when they are about four to six months old (Mombaerts *et al.* 1992).

A vaccine made with a chimeric VP6 protein and the mucosal adjuvant *E. coli* heat labile toxin LT(R192G) does not protect B6.129P2-*Tcrb*<sup>tm1Mom</sup>/J mice against murine rotavirus; however, it does

fully protect B6.129P2-*Tcr<sup>δ</sup>tm1Mom*/J and B6.129S2-*Igh-6<sup>tm1Cgn</sup>*/J mice. Furthermore, whereas the vaccine protects B6.129S2-*Igh-6<sup>tm1Cgn</sup>*/J mice depleted of CD8 T cells, it does not protect them if their CD4 T cells are depleted. Therefore, alpha/beta CD4 T cells seem to be the only lymphocytes required for protection (McNeal *et al.* 2002).

B6.129P2-*Tcrb*<sup>tm1Mom</sup>/J mice immunized with the well-defined T-dependent Ag, (4-hydroxy-3-nitrophenyl) acetyl (NP) conjugate, are able to induce Ig hypermutation. However, unlike the responding control B cells, which use canonical VDJ rearrangements, the responding B cells in these mice use analog genes of the J558 gene family. Additionally, the majority of anti-NP antibodies these mice produce use the kappa instead of the lambda light chain, the dominant chain used by mice of Igh(b) background. Thus, B cells that collaborate with gamma/delta T cells are distinct from those that interact with conventional alpha/beta Th cells (Zheng *et al.* 2003).

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## T-cell receptor-deficient mice *(cont.)*

### *Tcrd*

#### **B6.129P2-*Tcrd*<sup>tm1Mom</sup>/J (002120)**

B6.129P2-*Tcrd*<sup>tm1Mom</sup>/J homozygotes are viable and fertile. Gamma delta T-cell receptor expression is deficient in all adult lymphoid and epithelial organs. The alpha beta T-cell lineage develops normally. Patterns of CD4+CD8- and CD4-CD8+ alpha beta T-cells are apparently normal. These mice do not develop inflammatory bowel disease.

B6.129P2-*Tcrd*<sup>tm1Mom</sup>/J mice intratracheally inoculated with *Klebsiella pneumoniae* have a higher mortality rate than do either B6.129P2-

*Tcrb*<sup>tm1Mom</sup>/J mice or C57BL/6J controls. Although they have no trouble clearing the infection from their lungs, they do quickly upregulate IFNG and TNFA expression, and the infection spreads to peripheral blood and damages the liver. Thus, gamma delta-T cells are a critical part of the acute inflammatory response against extracellular Gram-negative bacteria and are vital for quickly producing the proinflammatory cytokines IFNG and TNFA (Moore *et al.* 2000).

### References

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### *Tcrb* and *Tcrd*

#### **B6.129P2-*Tcrb*<sup>tm1Mom</sup> *Tcrd*<sup>tm1Mom</sup>/J (002122)**

B6.129P2-*Tcrb*<sup>tm1Mom</sup> *Tcrd*<sup>tm1Mom</sup>/J homozygotes express neither alpha beta T-cell nor gamma delta T-cell receptors. Under certain housing conditions homozygotes develop mild inflammatory bowel disease.

Experiments using B6.129P2-*Tcrb*<sup>tm1Mom</sup> *Tcrd*<sup>tm1Mom</sup>/J mice reveal that clearing a primary rotavirus infection is B cell-mediated and T cell-independent. The Peyer's patches and

mesenteric lymph nodes of B6.129P2-*Tcrb*<sup>tm1Mom</sup> *Tcrd*<sup>tm1Mom</sup>/J, CD-1, and C57BL/6J mice infected with a homologous strain of murine rotavirus (EC wild type) are equally enlarged and contain comparable increases in the numbers activated B but not T cells. Three to four days after infection, the tissues of infected mice contain rotavirus-specific IgM but not IgA antibodies (Blutt *et al.* 2002).

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## Severely immunodeficient mice

Our major immunodeficient JAX® Mice models occur on several backgrounds (Table 2) and have either spontaneous or induced mutations in one or two genes (Table 3). Several are particularly useful for HIV/AIDS research. To choose the most appropriate model, you will have to consider many factors, including the following:

- **Background features.** Consider important features of the background strain, such as *H2* haplotype, behavior, and disease susceptibility. For example, the NOD/ShiLtJ background is susceptible to diabetes, and is deficient in natural killer (NK), macrophage, antigen presenting cell (APC), and complement activity (Tables 2, 4).
- **Functionality of various endogenous immune system components.** Consider the activity of the mutant's endogenous B cells, T cells, NK cells, APCs, and complement (Tables 2, 4).
- **Leakiness.** As applied to *Prkdc<sup>scid</sup>* mice, leakiness refers to their tendency (on certain backgrounds) to produce some functional B and T cells as they age. It is higher in mice housed under non-specific pathogen free (SPF) conditions, and it is generally high on the C57BL/6J and BALB/cByJ backgrounds, low on the C3H/HeSnJSmn background, and very low on the NOD/ShiLtJ background (Tables 2, 4).
- **Lifespan.** Some immunodeficient mice die young because they are susceptible to thymic lymphomas (Table 3). This limits their use for long-term experiments.
- **Radiosensitivity.** *Prkdc<sup>scid</sup>* mice are sensitive to radiation and therefore cannot be as thoroughly irradiated as other immunodeficient models before being engrafted (Table 4).
- **Breeding performance.** Some immunodeficient models, for example, NOD.Cg-*Prkdc<sup>scid</sup> B2m<sup>tm1Unc</sup>*/J, are difficult to breed (Table 4).
- **Gene features.** Consider how the gene of interest functions, and where it is expressed (Table 3).
- **Mutant gene effects.** Consider how the mutant gene affects immune responses (such as NK cell, macrophage, and complement activity) and interacts with the genetic background of a mutant. For example, the beta 2 microglobulin and perforin 1 (pore forming protein) mutations lower NK cell activity, the interleukin 2 receptor, gamma chain mutation completely eliminates it, and the *scid* mutation renders NOD mice resistant to diabetes (Tables 3, 4).
- **Availability.** Consider whether the mutant you want is readily available in the quantities you need.
- **Husbandry.** *Rag1<sup>null</sup>* and *Prkdc<sup>scid</sup>* mice should be housed in specific pathogen free (SPF) environments.
- **Research type.** Consider the kind of research you are conducting and how it will relate to previous and future research.

**Table 2.** Major features of selected backgrounds for immunodeficient JAX® Mice models and the names of selected models on those backgrounds (see Table 4 for model features).

Background	Background Features			Selected Immunodeficient JAX® Mice Models (stock number)
	Innate Immunity (NK, B, APC cells; complement activity)	<i>Scid</i> -associated leakiness	<i>H2</i> haplotype	
BALB Substrains	Normal	High	<i>d</i>	CBySmn.CB17- <i>Prkdc<sup>scid</sup></i> /J (001803) (commonly called BALB <i>scid</i> )
C57BL/6J	Normal	High	<i>b</i>	B6;129S7- <i>Rag1<sup>tm1Mom</sup></i> /J (002096) B6.129S7- <i>Rag1<sup>tm1Mom</sup></i> /J (002216) B6.CB17- <i>Prkdc<sup>scid</sup></i> /SzJ (001913)
NOD/ShiLtJ	Impaired	Low	<i>g<sup>7</sup></i>	NOD.129S7(B6)- <i>Rag1<sup>tm1Mom</sup></i> /J (003729) NOD.Cg- <i>Rag1<sup>tm1Mom</sup> Prf1<sup>tm1Sdz</sup></i> /SzJ (004848) NOD.CB17- <i>Prkdc<sup>scid</sup></i> /J (001303) (commonly called NOD <i>scid</i> ) NOD.Cg- <i>Prkdc<sup>scid</sup> B2m<sup>tm1Unc</sup></i> /J (002570) NOD.Cg- <i>Prkdc<sup>scid</sup> Il2rg<sup>tm1Wjl</sup></i> /SzJ (005557)

## Severely immunodeficient mice *(cont.)*

**Table 3.** Names and functions of genes mutated in selected immunodeficient JAX<sup>®</sup> Mice models.

<p><b><i>B2m</i></b> beta-2 microglobulin</p>	<p><i>B2m</i> is required for normal expression of major histocompatibility class I proteins (displaying viral and self antigens to potentially responsive T cells) and for CD8<sup>+</sup> T cell maturation and NK cell development</p>
<p><b><i>Il2rg</i></b> interleukin 2 receptor, gamma chain</p>	<p><i>Il2rg</i> is indispensable for IL2, IL4, IL7, IL9, IL15, and IL21 high affinity binding and signaling; in mice, it is also thought to play a key role in mediating susceptibility to thymic lymphomas. Thus, NOD.Cg-<i>Prkdc</i><sup>scid</sup> <i>Il2rg</i><sup>tm1Wjl</sup>/SzJ mice do not develop thymic lymphomas characteristic of aging NOD.CB17-<i>Prkdc</i><sup>scid</sup>/J mice. Most importantly, <i>Il2rg</i> deficiency blocks the development of NK cells and causes other defects in innate immunity.</p>
<p><b><i>Prf1</i></b> perforin 1 (pore-forming protein)</p>	<p><i>Prf1</i> is a critical component in the lytic pathway by which NK and CD8<sup>+</sup> lymphocytes kill targeted cells.</p>
<p><b><i>Prkdc</i></b> protein kinase, DNA-activated, catalytic polypeptide</p>	<p>The <i>scid</i> mutation in the <i>Prkdc</i> gene stands for severe combined immunodeficient. <i>Prkdc</i> is instrumental in repairing double-stranded DNA breaks and in recombining the variable (V), diversity (D), and joining (J) segments of immunoglobulin and T-cell receptor genes. Homozygous mutants have no mature T and B cells, cannot mount cell mediated and humoral adaptive immune responses, do not reject allogeneic and xenogeneic grafts, and are useful cancer research models. The <i>scid</i> mutation renders NOD mice diabetes-free and thereby makes them useful for adoptive transfer of diabetes by T cells. (Note: the NOD.NON-<i>Thy1</i><sup>a</sup>/1Lt (004483) strain provides an allotypically-marked T cell population and develops diabetes at the same rate and frequency as does the standard NOD/ShiLtJ (<i>Thy1</i><sup>b</sup>) strain. Thus, it is useful as a T cell donor source.)</p>
<p><b><i>Rag1</i></b> recombination activating gene 1</p>	<p><i>Rag1</i> is essential for the V(D)J gene rearrangements that generate functional antigen receptors in T and B cells; homozygous <i>Rag1</i><sup>tm1Mom</sup> mutation on the NOD background renders NOD mice diabetes-free. Aging NOD.129S7(B6)-<i>Rag1</i><sup>tm1Mom</sup>/J mice develop a high frequency of B cell and thymic lymphomas.</p>

The advantages and disadvantages of the most promising and widely used Immunodeficient JAX<sup>®</sup> Mice models are summarized in Table 4 (the following three pages).

## Severely immunodeficient mice (cont.)

**Table 4.** Major features of selected severely immunodeficient JAX<sup>®</sup> Mice models.

Model (stock number)	Lifespan (months)	Advantages	Disadvantages	Primary References
NOD.Cg-Prkdc <sup>scid</sup> Il2rg <sup>tm1Wjl</sup> /SzJ (005557)	>16	<p><b>Our most useful and versatile model</b></p> <ul style="list-style-type: none"> <li>no functional B and T cells</li> <li>no leakiness with age</li> <li>no NK cell activity</li> <li>lymphoma-resistant and long-lived</li> <li>enables long-term experiments</li> <li>superior ability to be humanized: excellent engraftment and differentiation of human hematopoietic stem cells (HSCs) into mature human lymphoid and myeloid cells</li> <li>superior for HIV and other infectious disease research: can essentially be engrafted with a human immune system</li> </ul>	<p><b>Few Disadvantages</b></p> <ul style="list-style-type: none"> <li>characterization in progress</li> </ul>	<p>Ishikawa <i>et al.</i> 2005; Shultz <i>et al.</i> 2005</p>
NOD.CB17-Prkdc <sup>scid</sup> /J (001303) (commonly called NOD scid)	8.5	<ul style="list-style-type: none"> <li>no functional B and T cells</li> <li>due to NOD background, low NK cell activity, no hemolytic complement activity, defects in myeloid development, and poor APC functions</li> <li>well characterized</li> <li>very low leakiness with age</li> <li>supports human hematolymphoid engraftment better than CBySmm.CB17-Prkdc<sup>scid</sup>/J or B6.CB17-Prkdc<sup>scid</sup>/SzJ</li> </ul>	<ul style="list-style-type: none"> <li>high incidence of thymic lymphomas (largely responsible for reduced lifespan)</li> <li>radiosensitive: tolerates up to 4 Gy</li> <li>suboptimal reconstitution with human HSCs</li> </ul>	<p>Shultz <i>et al.</i> 1995</p>
NOD.Cg-Rag1 <sup>tm1Mom</sup> Prf1 <sup>tm1Sdz</sup> /SzJ (004848)	8.5	<ul style="list-style-type: none"> <li>no functional B and T cells</li> <li>no leakiness with age</li> <li>no NK cell activity</li> <li>more lymphoma-resistant and longer-lived than NOD.Cg-Prkdc<sup>scid</sup> B2m<sup>tm1Unc</sup>/J</li> <li>radiation resistant: survive 8 Gy – optimizes radiation preconditioning for engraftment</li> <li>supports engraftment of human PBMCs and HSCs at about 10 fold higher levels than do NOD.CB17-Prkdc<sup>scid</sup>/SzJ and NOD.129S7(B6)-Rag1<sup>tm1Mom</sup>/J</li> <li>human PBMC engraftment results in high levels of CD4+ T cells and normalization of CD4:CD8 ratio</li> <li>engrafted human HSCs differentiate into myeloid, erythroid and B cell lineages, but not T cells</li> <li>breeds better than NOD.Cg-Prkdc<sup>scid</sup> B2m<sup>tm1Unc</sup>/J or NOD.CB17-Prkdc<sup>scid</sup>/SzJ mice</li> </ul>	<ul style="list-style-type: none"> <li>characterization in progress</li> <li>shorter lifespan than NOD.Cg-Prkdc<sup>scid</sup> Il2rg<sup>tm1Wjl</sup>/SzJ (due to thymic lymphomagenesis)</li> <li>less efficient reconstitution with human cells and tissues than NOD.Cg-Prkdc<sup>scid</sup> Il2rg<sup>tm1Wjl</sup>/SzJ</li> </ul>	<p>Shultz <i>et al.</i> 2003; Minamiguchi <i>et al.</i> 2005</p>

ND: not determined

## Severely immunodeficient mice (cont.)

**Table 4.** Major features of selected severely immunodeficient JAX<sup>®</sup> Mice models.

Model (stock number)	Lifespan (months)	Advantages	Disadvantages	Primary References
NOD.Cg-Prkdc <sup>scid</sup> -B2m <sup>tm1Unc</sup> /J (002570)	6.2	<ul style="list-style-type: none"> <li>no functional B and T cells</li> <li>very low leakiness with age</li> <li>does not express MHC class I molecules, resulting in virtually no NK cell activity</li> <li>supports engraftment of human PBMCs and HSCs at about 10 fold higher levels than do NOD.CB17-Prkdc<sup>scid</sup>/SzJ and NOD.129S7 (B6)-Rag1<sup>tm1Mom</sup>/J</li> <li>human PBMC engraftment results in higher levels of CD4+ T cells and normalization of CD4:CD8 ratio</li> <li>engrafts human HSCs that differentiate into myeloid, erythroid and B cell lineages, but not T cells</li> </ul>	<ul style="list-style-type: none"> <li>shortened lifespan due to thymic lymphomagenesis</li> <li>more radiosensitive than NOD.CB17-Prkdc<sup>scid</sup>/SzJ</li> <li>develops severe hemochromatosis</li> <li>PBMC engraftment results in high levels of T cells, but does not support increased levels of human B cell engraftment</li> <li>less efficient reconstitution with human cells and tissues than NOD.Cg-Prkdc<sup>scid</sup>/I2rg<sup>tm1Wfl</sup>/SzJ</li> </ul>	Christianson <i>et al.</i> 1997; Minamiguchi <i>et al.</i> 2005
NOD.129S7 (B6)-Rag1 <sup>tm1Mom</sup> /J (003729)	10.5	<ul style="list-style-type: none"> <li>no functional B and T cells</li> <li>no leakiness with age</li> <li>low NK cell activity</li> <li>more resistant to radiation and thymic lymphomas, and longer-lived than NOD.CB17-Prkdc<sup>scid</sup>/SzJ</li> <li>engrafts human lymphoid cells and HSCs at high levels</li> </ul>	<ul style="list-style-type: none"> <li>characterization in progress</li> <li>develops high frequency of pre-B cell lymphomas with age</li> </ul>	Shultz <i>et al.</i> 2000
B6.129S7-Rag1 <sup>tm1Mom</sup> /J (002216)	ND	<ul style="list-style-type: none"> <li>no functional B and T cells</li> <li>no leakiness with age</li> <li>for certain experiments, this strain is preferable: e.g., when it is necessary to match MHC with MHC restriction of a T cell receptor</li> </ul>	<ul style="list-style-type: none"> <li>high NK cell activity</li> <li>normal complement activity</li> <li>normal APC functions</li> </ul>	Mombaerts <i>et al.</i> 1992

ND: not determined

## Severely immunodeficient mice *(cont.)*

**Table 4.** Major features of selected severely immunodeficient JAX<sup>®</sup> Mice models.

Model (stock number)	Lifespan (months)	Advantages	Disadvantages	Primary References
B6.129S7-Rag1 <sup>tm1Mom</sup> /J (002096)	ND	<ul style="list-style-type: none"> <li>no functional B and T cells</li> <li>no leakiness with age</li> </ul>	<ul style="list-style-type: none"> <li>high NK cell activity</li> <li>normal complement activity</li> <li>normal APC functions</li> </ul>	Mombaerts <i>et al.</i> 1992
B6.CB17-Prkdc <sup>scid</sup> /SzJ (001913)	ND	<ul style="list-style-type: none"> <li>no functional B and T cells</li> </ul>	<ul style="list-style-type: none"> <li>very leaky with age</li> <li>elevated NK cell activity</li> <li>elevated complement activity</li> <li>normal APC functions</li> </ul>	Christianson <i>et al.</i> 1996
CBySmm.CB17-Prkdc <sup>scid</sup> /J (001803) (commonly called BALB Scid)	ND	<ul style="list-style-type: none"> <li>no functional B and T cells</li> <li>Incidence of thymomas about 3 fold less than NOD.CB17-Prkdc<sup>scid</sup>/SzJ</li> <li>for certain experiments, their MHC haplotype may be more appropriate than are those of NOD.CB17-Prkdc<sup>scid</sup>/SzJ</li> </ul>	<ul style="list-style-type: none"> <li>leaky with age</li> <li>normal complement activity</li> <li>normal numbers and functions of macrophages, NK cells, and APCs</li> <li>radiosensitive</li> <li>reduced lifespan due to thymic lymphomagenesis</li> <li>engraftment of human cells and tissues significantly less efficient than with NOD.CB17-Prkdc<sup>scid</sup>/SzJ, NOD.Cg-Prkdc<sup>scid</sup>B2m<sup>tm1Unc</sup>/J, and NOD.Cg-Rag1<sup>tm1Mom</sup>Prf1<sup>tm15dz</sup>/SzJ</li> </ul>	Custer <i>et al.</i> 1985

ND: not determined

## Severely immunodeficient mice *(cont.)*

***Foxn1***

**CByJ.Cg-*Foxn1*<sup>nu</sup>/J (000711)  
NU/J (002019)**

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Homozygotes for the nude spontaneous mutation (*Foxn1*<sup>nu</sup>, formerly *Hfh11*<sup>nu</sup>) have abnormal hair growth and develop defective thymic epithelium. Being athymic, they lack T cells and thus are incapable of cell-mediated immunity. However, their T-cell precursors are normal, and some adults produce some normal mature T cells. Their responses to thymus-dependent antigens (when detectable) are

primarily IgM. Homozygotes have a partial defect in B cell development, probably due to absence of functional T cells. Females are poor breeders: they begin to ovulate late (at 2.5 months) and stop early (at 4 months). NU/J mice, developed at The Jackson Laboratory, breed better than do CByJ.Cg-*Foxn1*<sup>nu</sup>/J. Nude mice have been used extensively in smallpox research (see selected abstracts in the smallpox section)

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### References

Committee on Immunologically Compromised Rodents 1989. Hereditary immunodeficiencies. Immunodeficient Rodents, a Guide to their Immunobiology, Husbandry, and Use, National Academy Press. pp 69-71.

## Toll like receptor-defective mice

*Tlr2*

**B6.129-*Tlr2*<sup>tm1Kir</sup>/J (004650)**

B6.129-*Tlr2*<sup>tm1Kir</sup>/J homozygotes are viable, and appear and behave normally. They produce no gene product (protein). Although bone marrow-derived macrophages do not respond to *Borrelia burgdorferi* lipoprotein challenge, they are activated by non-lipoprotein sonicate. Arthritis due to *B. burgdorferi* infection is more severe in mutants than in controls. Tissues of infected mutants can contain up to 100 times more bacteria than do those of wild-type littermates. Elevated spirochete numbers persist eight weeks post-infection. Homozygotes produce neither TNFA nor IL6 and do not become ill when treated with *Leptospira interrogans* LPS. This strain may be useful for studying responses to bacterial endotoxins (Wooten *et al.* 2002).

Experiments using BALB/cJ, C57BL/6J, C.C3-*Tlr4*<sup>Lps-d</sup>/J, *Tlr2*-deficient, and *Tnf*-deficient mice revealed that TLR2 plays a critical role in the ability of dendritic cells to produce IL6 and IL10 in response to an *M. tuberculosis* infection. In contrast, neither TLR2 nor TLR4 are necessary for IL12 production. Additionally, neither TLR2 nor TLR4 affects dendritic cell maturation and their ability to influence the polarity of differentiating naive T cells. These results may help researchers design vaccines based on regulating IL6 and IL10 in dendritic cells (Jang *et al.* 2004).

Even after being infected with high doses of

*L. monocytogenes*, *Tlr2*-, *Tlr4*-, and *MyD88*-deficient mice can generate *Listeria*-specific CD8+ and CD4+ Th1 responses sufficient to control secondary infection (Kursar *et al.* 2004).

Experiments with *Tlr2*- and *Tlr4*-deficient mice revealed that innate immune cells need TLR2 but not TLR4 to recognize hepatitis C virus (HCV) core and NS3 proteins. This capability is not augmented by co-expression of the TLR co-receptor CD14. These data help demonstrate that plasmacytoid and myeloid dendritic cell functions in patients with chronic HCV infection are impaired, and that dendritic cell defects are likely related to interaction of HCV viral products with innate immune cells (Szabo and Dolganiuc 2005).

Whereas *Tlr2*- and *MyD88*-deficient mouse pups cannot prevent the spread of a local and low dose infection of group B streptococcus (GBS), all infected controls can. Bacterial burden is higher in *MyD88*- than in *Tlr2*-deficient mice. In contrast, a high bacterial dose induces high bacteremia both in mutants and controls. Under these conditions, either TLR2 or *MyD88* deficiency significantly protects mice from death, concomitantly with decreased circulating levels of TNFA and IL6. Thus, TLR2 and *MyD88* have a dual role in the host defense against GBS sepsis, and TNFA is likely the molecular mediator of bacterial clearance and septic shock (Mancuso *et al.* 2004).

## References

- Jang S, Uematsu S, Akira S, Salgame P. 2004. IL-6 and IL-10 induction from dendritic cells in response to *Mycobacterium tuberculosis* is predominantly dependent on TLR2-mediated recognition. *J Immunol* 173:3392-7.
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## Toll like receptor-defective mice *(cont.)*

### *Tlr3*

#### **B6;129S1-*Tlr3*<sup>tm1Flv</sup>/J (005217)**

B6;129S1-*Tlr3*<sup>tm1Flv</sup>/J homozygotes are viable, fertile, and appear and behave normally. They produce a truncated non-functional gene product. When challenged with poly(I:C), polyinosine-polycytidylic acid, a synthetic dsRNA analog, their macrophages produce no inflammatory cytokines, IFNG, and IFNB. They are resistant to poly(I:C) induced shock and produce reduced levels of IL12. Their splenocytes do not respond to viral dsRNA and produce reduced quantities of IL6.

After being infected with West Nile virus (WNV), *Tlr3*-deficient mice have fewer peripheral

cytokines, a greater peripheral but lower brain viral load, and less brain inflammation and damage than do infected controls. Peripheral infection in infected controls breaks down the blood-brain barrier and enhances brain infection. Infected *Tlr3*-deficient mice and controls are equally susceptible to intracerebroventricular WNV infections. *Tnfr1*-signaling is vital for blood-brain barrier compromise (Wang *et al.* 2004).

This strain is currently under development for distribution. To register your interest, contact our customer support department at [orderquest@jax.org](mailto:orderquest@jax.org)

### References

- Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. 2003. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature* 413:732-8.
- Wang T, Town T, Alexopoulou L, Anderson JF, Fikrig E, Flavell RA. 2004. Toll-like receptor 3 mediates West Nile virus entry into the brain causing lethal encephalitis. *Nat Med* 10:1366-73.

### *Tlr4*

#### **C3H/HeJ (000659)**

C3H/HeJ is a general-purpose strain used for a wide variety of research, including cancer, immunology and inflammation, sensorineural, and cardiovascular biology. A spontaneous mutation, *Tlr4*<sup>ps</sup>, in the toll-like receptor gene of the C3H/HeJ strain renders it endotoxin-resistant. Consequently, it is highly susceptible to infection by

Gram-negative bacteria such as *Salmonella enterica*. When infected with *Salmonella*, C3H/HeJ mice exhibit delayed chemokine production, impaired NO generation, and attenuated cellular immune responses. Infected mice seem to die from enhanced bacterial growth within the liver Kupffer cell network (Vazquez-Torres *et al.* 2004).

### References

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- Vazquez-Torres A, Vallance BA, Bergman MA, Finlay BB, Cookson BT, Jones-Carson J, Fang FC. 2004. Toll-like receptor 4 dependence of innate and adaptive immunity to *Salmonella*: importance of the Kupffer cell network. *J Immunol* 172:6202-8.

## Toll like receptor-defective mice *(cont.)*

***Tlr4***

**C57BL/10ScNJ (003752)**

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These mice have a deletion of the *Tlr4* gene (*Tlr4<sup>Lps-del</sup>* – different from the *Tlr4<sup>Lps-d</sup>* mutation of C3H/HeJ) and therefore do not respond to LPS. The allele for normal LPS response, *Tlr4<sup>Lps-n</sup>*, occurs

in most other C3H and C57BL/10 substrains and most other mouse strains.

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### References

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***Tlr4***

**C.C3-*Tlr4<sup>Lps-d</sup>*/J (002930)**

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In addition to the *Tlr4<sup>Lps-d</sup>* congenic interval from C3H/HeJ, this strain is congenic for the wild-type tyrosinase allele from C3H/HeJ on chromosome 7. This strain is a tool for analyzing markers in the region of and for examining functional effects of *Lps<sup>d</sup>* on BALB/c, a strain susceptible to infection and neoplastic disease, including the induction of plasmacytomas and other tumors (Andonegui *et al.* 2003).

Experiments using C.C3-*Tlr4<sup>Lps-d</sup>*/J, C.129S7(B6)-*Ifng<sup>tm1Ts</sup>*/J, C.129S1-*Il12rb1<sup>tm1Jm</sup>*/J, and BALB/cJ mice infected with Coxsackievirus B3 (CB3) revealed remarkable similarities

between the effects of IL12RB1 and TLR4: each significantly increases levels of myocarditis, viral replication, and levels of IL1B and IL18. In contrast, IFNG decreases viral replication and inflammation in the heart. The similar effects of IL12RB1 and TLR4 suggest that they share common downstream pathways that directly influence the production of IL1B and IL18, which in turn play a significant role in CB3-induced myocarditis. Similar dynamics may be involved in other autoimmune diseases triggered by viral infections (Fairweather *et al.* 2003).

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Fairweather D, Yusung S, Frisancho S, Barrett M, Gatewood S, Steele R, Rose NR. 2003. IL-12 receptor beta 1 and Toll-like receptor 4 increase IL-1 beta- and IL-18-associated myocarditis and coxsackievirus replication. *J Immunol* 170:4731-7.

## Vaccine development

### HLA-A

#### C57BL/6-Tg(HLA-A2.1)1Enge/J (003475)

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Homozygotes for the Tg(HLA-A2.1)1Enge transgene express significant quantities of the human class I MHC Ag HLA-A2.1 on their spleen, bone marrow, and thymus cells. This expression does not result in expansion of the number of cytotoxic T lymphocyte (CTL) precursors specific for either other human class I Ag, HLA-B27, or HLA-A2.2. These mice have been used to identify

hepatitis C virus (HCV) peptides expressing a sequence for HLA-A2.1 binding that are actually recognized by human A2.1-restricted CTLs. Thus, they are important for the study of HLA-restricted CTL determinants and for developing vaccines against HCV. (Copy number may vary. Hemizygotes are tested for expression before they are distributed.)

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Le A-XT, Bernhard EJ, Holterman MJ, Strub S, Parham P, Lacy E, Engelhard VH. 1989. Cytotoxic T cell responses in HLA-A2.1 transgenic mice. *J Immunol* 142:1366-71.

### HLA-A/H2-D

#### B6.Cg-Tg(HLA-A/H2-D)2Enge/J (004191)

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Homozygotes for the Tg(HLA-A/H2-D)2Enge transgene are viable, fertile, and look and behave normally. They express an interspecies hybrid class I MHC gene, AAD, which contains the alpha-1 and alpha-2 domains of the human HLA-A2.1 gene and the alpha-3 transmembrane and cytoplasmic domains of the mouse H-2D<sup>d</sup> gene, under the direction of the human HLA-A2.1 promoter. The HLA-A2.1 recombinant transgene is expressed at the same level as are the endogenous mouse class I molecules. The mouse alpha-3 domain expression enhances the immune response. Compared to unmodified HLA-A2.1, the chimeric HLA-A2.1/H2-D<sup>d</sup> MHC Class I

molecule mediates efficient positive selection of mouse T cells to provide a more complete T cell repertoire capable of recognizing peptides presented by HLA-A2.1 Class I molecules. The peptide epitopes presented and recognized by mouse T cells in the context of the HLA-A2.1/H2-D<sup>d</sup> class I molecule are the same as those presented in HLA-A2.1+ humans. This strain models human T cell immune responses to HLA-A2 presented antigens, and can be used to identify those antigens. It is an important preclinical model for designing and testing vaccines for infectious diseases involving optimal stimulation of Cd8+ cytolytic T cells.

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### References

Newberg MH, Smith DH, Haertel SB, Vining DR, Lacy E, Engelhard VH. 1996. Importance of MHC class I alpha2 and alpha3 domains in the recognition of self and non-self MHC molecules. *J Immunol* 156:2473-80.

## Macroarrays

In the Winter 2004 issue of JAX<sup>®</sup> Notes (JAX<sup>®</sup> NOTES 2005), we introduce the concept of the “mouse macroarray” and define it as a set of Mouse Phenome Project (MPP) inbred strains that can be used to comprehensively characterize a response to a chemical (pharmaceutical drug, toxin, alcohol, etc.). In a recent “Macroarray Resource Manual” (available free by filling out the literature request form at [www.jax.org/jaxmice/literature](http://www.jax.org/jaxmice/literature)), we summarize several studies that illustrate applications of the original macroarray

concept, and we expand the concept to include sets of chromosome substitution (CS) strains, recombinant inbred (RI) strains, and recombinant congenic (RC) strains that can be used in quantitative trait locus (QTL) analyses and gene discovery. In this manual, we further expand the macroarray concept to include sets of inbred mouse strains that may be used to characterize the pathogenesis of, immune response to, and the efficacy of therapies and vaccines against infectious diseases.

### Phenome Strains

The Mouse Phenome Strains were selected by the Steering Committee of the Mouse Phenome Project (MPP, [www.jax.org/phenome](http://www.jax.org/phenome)), a coordinated international effort to collect and make publicly available baseline phenotypic data

for a set of 40 commonly used and genetically diverse inbred mouse strains. The 40 strains were placed into four priority groups (Table 1): A, B, C, and D, group A having the highest priority.

**Table 5.\*** Classification of MPP strains according to priority

Group A** (10)	Group B** (10)	Group C** (10)	Group D** (10)
129S1/SvImJ	AKR/J	BUB/BnJ	BTBR <i>T<sup>+</sup> tf/J</i>
A/J	C57L/J	C57BL/10J	C57BR/cdJ
BALB/cByJ	C58/J	C57BLKS/J	CE/J
C3H/HeJ	MOLF/EiJ	CBA/J	I/LnJ
C57BL/6J	NOD/ShiLtJ	CZECHII/EiJ	JF1/Ms
CAST/EiJ	NZB/BINJ	KK/HlJ	MA/MyJ
DBA/2J	PERA/EiJ	LP/J	NON/ShiLtJ
FVB/NJ	PL/J	MSM/Ms	NZW/LacJ
SJL/J	SM/J	RIIIS/J	PWK/PhJ
SPRET/EiJ	SWR/J	WSB/EiJ	SEA/GnJ

\*This strain list is subject to revision by the MPP Steering Committee.

\*\* Groups A and B include strains suitable for all types of research, many commonly used strains, strains with extensive sequence data (JAX<sup>®</sup> Mice strains C57BL/6J, 129S1/Sv1mJ, DBA/2J, and A/J), and those to be sequenced in the NIEHS-supported Resequencing Project (JAX<sup>®</sup> Mice strains 129S1/SvImJ, A/J, AKR/J, BALB/cByJ, BTBR *T<sup>+</sup> tf/J*, C3H/HeJ, CAST/EiJ, DBA/2J, FVB/NJ, MOLF/EiJ, KK/HlJ, NOD/ShiLtJ, NZW/LacJ, PWD/PhJ, and WSB/EiJ) ([www.niehs.nih.gov/oc/news/micedna.htm](http://www.niehs.nih.gov/oc/news/micedna.htm)), progenitors of either transgenesis or mutagenesis studies, progenitors of recombinant inbred, congenics, and chromosome substitution strains, are generally easy to breed and maintain, and are genetically diverse (include wild-derived *Mus* subspecies). Groups C and D include strains with additional genetic diversity, and strains of special interest.

## Macroarrays

Data collected by the MPP is organized and housed in the Mouse Phenome Database (MPD, [www.jax.org/phenome](http://www.jax.org/phenome)) at The Jackson Laboratory. The MPD is linked with the Mouse Genome Database (MGD, [www.informatics.jax.org](http://www.informatics.jax.org)) and other relevant and publicly accessible databases. Some of the phenotypic data in the MPD include: baseline and diet-induced levels of lipids, lipoproteins, glucose, and hormones; body

weight, body composition, and bone mineral density and content; susceptibility to diseases such as gallstones and atherosclerosis; susceptibility to mammary tumors; *H2* haplotypes; and anthrax susceptibility. They are characterized by strain and sex. Investigators may download protocols and either raw or summary data, and analyze data using MPD tools.

## Recombinant Inbred (RI) Strains

The AXB (16 strains) and BXA (14 strains) RI strains differ in their susceptibility to infectious diseases. RI strain distribution patterns for all RI

sets distributed by The Jackson Laboratory can be found at [www.informatics.jax.org/searches/riset\\_form.shtml](http://www.informatics.jax.org/searches/riset_form.shtml) Form.

## References

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## Annual Workshop on the Pathology of Mouse Models for Human Disease

This week-long intensive workshop in pathology and histopathology includes didactic sessions on particular disease areas and models. Participants interact with prominent mouse pathologists and geneticists from leading research institutions..

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- Concepts of mouse models and immune system pathology
- Pathology of infectious diseases, hematopoietic and respiratory systems, and gene expression analysis
- Comparative histopathology, mouse phenotyping, pathology of embryonic development and ophthalmic systems
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*For more information on this and other Jackson Laboratory courses and conferences, see our Courses and Conferences Web site: [www.jax.org/courses/events/current.do](http://www.jax.org/courses/events/current.do)*

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“The capacity to blunder slightly is the real marvel of DNA. Without this special attribute, we would still be anaerobic bacteria and there would be no music.”



— Lewis Thomas (1913-1993)  
*Physician, Science Writer*

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