1 Mouse

INTRODUCTION

Apart from the many inbred strains, substrains, spontaneous mutants, and outbred stocks of laboratory mice, the mouse has been, and continues to be, central to molecular genomics, with worldwide efforts continuing to "knock out" every functional gene in the mouse genome and define the relationship between genotype and phenotype. In addition to understanding the genome, various mouse strains and stocks, as well as the genetically engineered mouse (GEM), play critical roles in hypothesis-driven biomedical research. These trends have created rich opportunities and critical demand for comparative pathologists who are knowledgeable in mouse pathobiology. Unfortunately, the scientific literature is replete with erroneous interpretation of phenotype by scientists (as well as pathologists) lacking expertise in mouse pathology. Effective mouse pathology requires a global understanding of mouse biology, euphemistically termed "Muromics" (see Barthold 2002).

It is impossible for the pathologist to command in-depth knowledge of all strains, stocks, and mutant types of mice, and in many cases there is little baseline data to draw upon. Nevertheless, the mouse pathologist must be cognizant of general patterns of mouse pathology, as well as strain- and GEM-specific nuances. Recommended references (Frith & Ward, 1988; Maronpot et al. 1999; McInnes 2012; Mohr 2001; Mohr et al. 1996; Ward et al. 2000) provide thorough pictorial coverage of spontaneous mouse pathology in several common inbred strains of mice. The incidence and prevalence of strain-specific pathology are highly dependent upon genetic and environmental influences, including diet, bedding, infectious disease, age, sex, and other factors. Compared to the above-cited references, our coverage of the esoterica of spontaneous mouse pathology is relatively superficial. We herein emphasize general patterns of disease, while attempting to address important strain-, mutant-, and GEM-specific diseases when appropriate. There are a growing number of internet-accessible resources for mouse phenotyping and pathology of strains, stocks, and GEMs. A listing of these web resources is available through various sources (Bolon 2006; Brayton 2013; Fox et al. 2007, 2015). Although not specifically listed in this text, it is worth "surfing" through these cited websites that provide a plethora of information at multiple levels.

The unique qualities of the laboratory mouse and the precision of mouse-related research make infectious agents, even those with minimal (or no) pathogenicity, major concerns due to their potential and sometimes significant impact upon research reproducibility, including phenotype. A challenge that is unique to the mouse is the difficulty in drawing the line between commensalistic, opportunistic, or overtly pathogenic microorganisms. Since the last edition, a wide variety of immune-deficient GEMs have been created, thereby raising the status of several relatively innocuous infectious agents to the level of pathogens. Immune-deficient mice and new molecular methods of detection continue to reveal previously unrecognized mouse "pathogens," such as a number of *Helicobacter* spp., norovirus, and most recently, astrovirus. Furthermore, the unrestricted traffic of GEMs among institutions and the pressure to reduce costs of maintenance at the expense of quality control have resulted in the re-emergence of several infectious agents that have not been seen in several decades. We, therefore, unabashedly emphasize mouse infectious diseases in this chapter. Despite advances in husbandry and diagnostic surveillance, we are reluctant to discard entities that may seem to have disappeared from laboratory mouse populations because of their likelihood of return.

Pathology of Laboratory Rodents and Rabbits, Fourth Edition. Stephen W. Barthold, Stephen M. Griffey, and Dean H. Percy.

MOUSE GENETICS AND GENOMICS

The laboratory mouse is an artificial creation, and there is no true "wild-type" laboratory mouse. Furthermore, there is no such thing as "normal" microflora, since laboratory mice are often maintained in microbially pristine environments devoid of pathogens and opportunistic pathogens, as well as other commensal flora/fauna. Laboratory mice are largely derived from domesticated "fancy mice" that arose from many years of trading mouse variants among fanciers in Europe, Asia, North America, and Australia. The laboratory mouse genome is, therefore, a mosaic derived from different subspecies of the *Mus musculus* (house mouse) complex, including *M.m. domesticus*, *M.m. musculus*, *M.m. castaneus*, *M.m. molossinus* (a natural hybrid of *M.m. musculus* and *M.m. castaneus*), and others. The genome of *M.m. domesticus* is the predominant contributor to most strains of mice, but many inbred strains share a common "Eve" with a mitochondrial genome of *M.m. musculus* origin and a common "Adam" that contributed their Y chromosome from *M.m. castaneus*. In addition, there is evidence that other *Mus* species, outside of the *M. musculus* complex, have contributed to the genome of some, but not all, laboratory mouse strains. For example, the C57BL mouse genome contains a contribution from *M. spretus*. Perhaps the only laboratory mouse that is derived from a single *M.m. domesticus* species (subspecies) is the "Swiss" mouse. Several *Mus* species that are outside of the *M. musculus* complex, such as *M. spretus*, have been inbred. Thus, the laboratory mouse genome is not uniform among strains and mouse strains are not entirely within the *M. musculus* clade.

There are over 450 inbred strains of laboratory mice that have arisen during the last century, and these strains, which were selectively inbred to pan-genomic homozygosity for purposes entirely unrelated to modern research, are the foundation upon which literally thousands of spontaneous mutants and GEMs have been built. Additional inbred strains have been developed from wild mice (*M.m. castaneus*, *M. spretus*, etc.). Furthermore, "outbred" mice (mostly Swiss mice) are highly homozygous and nearly inbred. In addition to historical inbreeding that may be intentional or the inadvertent result of maintaining small populations of mice, rederivation of a mouse population results in genetic bottlenecks as well. There is no such thing as a truly "outbred" laboratory mouse with a fully heterozygous genome representative of wild-type *M. musculus*, and there is no wild mouse genetic counterpart of the laboratory mouse. Recently, an octaparental Diversity Outbred (DO) mouse stock was developed from eight disparately related inbred strains of mice, but this stock is not extensively utilized. When working with mice, the pathologist must also become facile with strains, substrains, sub-substrains, hybrids, congenics, insipient congenics, coisogenics, consomics, conplastics, recombinant inbreds, recombinant congenics, spontaneous mutants, random induced (radiation, chemical, retroviral, gene trap) mutants, transgenics (random insertions), and targeted mutant mice, each with relatively unique, predictable, and sometimes unpredictable phenotypes and patterns of disease whose expression is modified by environmental and microbial variables.

The inherent value of the laboratory mouse is its inbred genome, but maintaining the genetic stability of inbred strains of mice is a challenge. Since the advent of GEMs, there has been widespread genetic mismanagement of mouse strains by investigators with considerable skill in mouse genomics but limited expertise in mouse genetics. Even with the best of intentions, continuous inbreeding leads to substrain divergence among different populations of the same parental origin due to spontaneous mutations, retrotransposon integrations, or residual heterozygosity. Genetic contamination is also a surprisingly frequent event in both commercial and academic breeding colonies of mice. Within a few generations, substrain divergence can result in significant differences in phenotype, including response to research variables. The variable genetic contributions of different origins of mice and selective inbreeding for strain characteristics, such as coat color or neoplasia, are especially important when considering retroelements, which make up 37% of the mouse genome. Retroelements are highly dynamic within the context of the inbred mouse genome. They are present in the genomes of all mammals but have become artificially important in the homozygous genome of the laboratory mouse, and in fact had much to do with development of original inbred strains of mice with unique phenotypes, especially coat color and neoplasia. It is difficult to ignore their impact on mouse pathology, and thus retroelements are discussed later in this chapter (see Section "Retroelements and Retroviral Infections").

NOMENCLATURE

The details of mouse nomenclature are beyond the scope of this book, but it is critically important that the full and correct strain, substrain, and mutant allelic or transgene nomenclature be utilized when evaluating pathology and in publications for maximal reproducibility of results. Being able to "read" the nomenclature of a mouse that is submitted for evaluation is critical for interpreting pathology. Guidelines for mouse nomenclature are available at the *International Mouse Nomenclature* home page (http://www.informatics.jax.org/mgihome/nomen/). *The Mouse in Biomedical Research: History, Genetics, and Wild Mice* (Fox et al. 2007), the mouse chapter in *Laboratory Animal Medicine* (Fox et al. 2015), and *Mouse Genetics* (L.M. Silver 1995) are also useful sources of information on mouse genetics, genomics, and nomenclature.

COMMON INBRED STRAINS

Among the many inbred strains, the great majority of biomedical research, including genomic research, is based on a relatively few mouse strains, including C57BL/6, BALB/c, C3H/He, 129, FVB, and outbred Swiss stocks. This is fortuitous for the pathologist, as familiarity with this relatively small list of strains provides a good basis for approaching the general pathology of mice. Despite emphasis on mouse strains, there are significant genotypic and phenotypic differences among substrains of any given strain, such as C57BL/6J versus C57BL/6N and among the various strains of 129 mice. An overview of characteristics among inbred strains has been developed by Festing (http://www.informatics.jax.org/external/festing/mouse/STRAINS.shtml) and The Mouse Phenome Database provides comprehensive information on many strains of mice (http://www.phenome.jax.org).

The reader is referred to other sources for more comprehensive information regarding the myriad possibilities of background pathology among laboratory mice (see Section "General References on Diseases of Mice"). This text is not intended to provide such depth of coverage, but herein provides a brief synopsis of important disease characteristics of the major strains/stocks of mice. The specific lesions are described further in later sections of this chapter.

C57BL/6 (B6) mice are the gold standard "background strain" for GEMs created by homologous recombination. Many mutant alleles and transgenes are backcrossed onto this strain. There are a number of other related "black" strains, including C57BL/10 (B10). B6 mice were initially bred for their longevity. Their melanism is manifested by their coat color, as well as melanin pigment in heart valves, splenic capsule and trabeculae, meninges, cerebral vessels, Harderian glands, and parathyroid glands. Common strain-related spontaneous diseases include hydrocephalus, hippocampal neurodegeneration, microphthalmia and anophthalmia, age-related cochlear degeneration and hearing loss, and malocclusion. B6 mice are predisposed to barbering or trichotillomania, which renders them susceptible to alopecia and staphylococcal ulcerative dermatitis. Aged B6 mice develop acidophilic macrophage pneumonia and epithelial hyalinosis, which are rapidly accelerated in B6 mice with the moth-eaten and various other mutations. B6 mice may develop late-onset amyloidosis, but this is highly dependent upon environmental and infectious factors (e.g., dermatitis). The most common B6 neoplasms are lymphoma, hemangiosarcoma, and pituitary adenoma.

BALB/c mice (BALB/c, BALB/cBy, et al.) are albinos. Mature males are rather pugilistic, requiring separate housing for particularly fractious individuals. Dystrophic epicardial mineralization of the right ventricular free wall is common, and they are prone to development of myocardial degeneration and auricular thrombosis. Corneal opacities are commonly found, and they often develop conjunctivitis, blepharitis, and periorbital abscesses. Hypocallosity (corpus callosal aplasia) is frequent, and they develop age-related hearing loss. BALB mice are remarkably resistant to spontaneous amyloidosis, in contrast to other mouse strains. The livers of normal BALB mice feature a moderate amount of hepatocellular fatty change. The most common tumors of BALB mice are pulmonary adenomas, lymphomas, Harderian gland tumors, and adrenal adenomas. Myoepitheliomas of salivary, preputial, and other exocrine glands are also relatively common in this strain.

C3H/He mice are agouti mice that are blind due to *rd1* mutation (*Pde6b*^{rd1}) and are also prone to corneal opacities and hearing loss later in life. They frequently develop focal myocardial and skeletal mineralization and myocardial degeneration. C3H/HeJ mice develop alopecia areata as they age. They are susceptible to exogenous murine mammary tumor virus (MMTV)-induced mammary tumors and develop a relatively high incidence of mammary neoplasia later in life due to endogenous MMTV. Other relatively common tumors include hepatocellular tumors.

129 mice rank high in the panoply of mousedom as the most frequent source of embryonic stem (ES) cells, from which most targeted mutant mice are derived. The 129 mouse is not a single strain, and in fact "129" is represented by 16 recognized strains and substrains. This is due to accidental and intentional genetic contamination of the original 129 strain by various laboratories. Thus, the designation 129 is followed by P, S, T, or X, and other designations, in addition to substrain determinants. Genetic differences between the targeting construct and the ES cells can significantly influence efficiency of homologous recombination. The differences among 129 mice are not subtle, with variation in coat color, behavior, and other characteristics, including patterns of pathology. Hypocallosity is relatively common in many 129 mice. 129 mice, like B6 mice, are prone to pulmonary proteinosis and epithelial hyalinosis. Megaesophagus occurs in some types of 129 mice. Blepharitis and conjunctivitis are common in 129P3 mice. 129/Sv mice are renown for development of testicular teratomas (aka embryonal carcinomas). Other common neoplasms in 129 mice are lung tumors, Harderian gland tumors, ovarian tumors, and hemangiosarcomas.

FVB/N mice are inbred Swiss mice that gained popularity for creation of transgenic mice in an inbred genetic background. They are blind due to homozygosity of *rd1* allele (*Pde6b*^{rd1}) and prone to seizures. Many lines of FVB mice develop persistent mammary hyperplasia and hyperplasia or adenomas of prolactin-secreting cells in the anterior pituitary, but mammary tumors are rare (unless through transgenesis). Common neoplasms include tumors of lung, pituitary, Harderian gland, liver, lymphomas, and pheochromocytomas.

NOD mice are inbred Swiss mice that were selectively bred for cataracts, and during that process were found to develop type 1 diabetes (nonobese diabetes (NOD)). This strain develops a number of other autoimmune disorders that are genetically determined at multiple loci. Notably, they have functional defects in macrophage and dendritic cell function, NK cells, NKT cells, regulatory CD4+CD25+ cells, and are C5a deficient. Their susceptibility to diabetes is highest when they are maintained in relatively germ-free environments, and is much lower in conventional environments. The NOD strain was genetically modified through backcrossing to create a xenotransplant host that is globally defective in NK cells, macrophage and dendritic cells (NOD characteristics), T and B cells (*Prkdc*^{scid}), and IL-2-receptor γ (*IL-2rγ*^{tm1Wjl}). The resultant strain, NOD.Cg*Prkdc*^{scid}*IL2rγ*^{tm1Wjl}/SvJ (*NSG*) has become the optimal host for xenogeneic transplants, particularly human stem cell and T-cell engraftment. As a result, graft versus host disease (GVHD) arises in engrafted mice, characterized by human T-cell infiltration of skin, liver, intestine, lungs, and kidneys (see discussion of GVHD in Section "Aging, Degenerative, and Miscellaneous Disorders"). Because of their global immunodeficiency, mice of this strain are uniquely susceptible to opportunistic infections.

Outbred Swiss mice are all closely related derivatives of a small gene pool of founder animals that were inbred for many generations in various laboratories before outbreeding, primarily by commercial vendors. Outbred Swiss mice are often erroneously considered "wild-type" for comparison with inbred mice. As noted previously, they are far from outbred and differ genetically from inbred mice. Many, but not all, Swiss mouse stocks have retinal *rd1* degeneration (homozygous recessive), reflecting their high degree of homozygosity. Swiss mice are particularly prone to amyloidosis, which is a major life-limiting disease. They develop a variety of incidental lesions, and the most common tumors are lymphomas, pulmonary adenomas, liver tumors, pituitary adenomas, and hemangiomas/sarcomas, among others.

GENOMIC CONSIDERATIONS FOR THE PATHOLOGIST

Having stressed the importance of strain and substrain, it is notable that the mouse genomic community does not utilize a single strain of mouse, and when they do use a similar strain, it is often a different substrain. GEMs are created in a variety of ways, including random mutagenesis (chemical mutagenesis, radiation, random transgenesis, gene trapping, retroviral transgenesis) and targeted mutagenesis (homologous recombination). Issues relevant to the pathologist with the most common means of creating GEMs, random transgenesis and targeted mutations, are discussed below.

Random insertion of transgenes is accomplished through pronuclear microinjection of zygotes with ectopic DNA (transgenes). This has generally been achieved using hybrid zygotes of 2 inbred parental strains, outbred Swiss mice, or from inbred Swiss FVB/N mice to take advantage of hybrid vigor to compensate for the trauma of microinjection and facilitate the process of microinjection by providing large pronuclei. Transgenes become randomly integrated throughout the genome, often in tandem repeats, so that each pup within a litter arising from microinjected zygotes is hemizygous for the transgene, but is genetically distinct from its littermates. The degree of transgene expression (phenotype) varies with the location of the transgene within the genome. Each founder line of the same transgene represents a unique and nonreproducible genotype and, therefore, phenotype. Transgenes tend to be genetically unstable, and copies may be lost in subsequent generations, resulting in ephemeral phenotypes. Transgene insertions can also lead to unanticipated altered function of genes through insertional mutagenesis, or regulation by flanking genes within the area of insertion. Unanticipated phenotypes, such as immunodeficiency or other effects, can therefore occur. The use of hybrids or outbred mice as founders requires selective inbreeding to attain a useful model. This can be circumvented by using inbred founders, such as FVB/N mice. Maintaining the transgene on an outbred genetic background or incompletely backcrossed background poses problems with uncontrolled modifier and compensatory genes that may unpredictably influence phenotype.

The discipline of mouse genomics has lent itself to incredible precision through homologous recombination, with the ability to alter not only specific genes but also gene function at specific time points during development or life stage, create tissue-specific gene alterations, gain of function, loss of function, and targeted integration of transgenes that allow customized development of mouse models of human disease that would not ordinarily arise within the context of the indigenous mouse genome. Targeted mutant mice are often created in one of several types of 129 ES cells, and once germline transmission has been effected, the 129-type mutant mouse is usually backcrossed to a more utilitarian mouse strain, such as B6. Full backcrossing to congenic status requires 3–4 years, which is seldom fulfilled. In constructs that require cre-lox technology, mutant mice are further crossed with cre transgenic mice, which may be of another strain, substrain, or stock background. Thus, despite superb precision in altering a gene of interest, the rest of the mouse's genome can remain highly heterogeneous, which defeats the inherent value of the GEM for research, or at least limits its full potential.

ES cells, and the mutations that they carry, are most often derived from one of the 129-type mouse strains, and ES cells become mice through the generation of chimeric progeny. Insufficient backcrossing, with retention of 129 characteristics, may result in erroneous

assumptions about the phenotype of the targeted gene. There is considerable genetic variation among different 129 ES cell lines, which can be a potential problem for comparing phenotypes of the same gene alteration among different 129 ES cell-derived mice. The process of creating chimeric mice, which is an essential step involving microinjection of 129 ES cells into a recipient blastocyst, has consequences. Most ES cell lines are "male" (XY), but blastocysts are either male or female. Hermaphroditism is quite common in chimeric mice arising from XY and XX cells. XX/XY chimeras are usually phenotypically male, but may have testicular hypoplasia and lower fertility. XX/XY chimeras may also have cystic Muellerian duct remnants, an ovary and a testis, and/or ovotestes. In addition to gonadal teratomas that are inherent in many 129 mouse strains, extragonadal teratomas arising from 129 cells in chimeric mice can develop in perigenital regions and the midline.

Because of the highly inbred nature of laboratory mice, experimental mutation of many genes often leads to embryonic or fetal death that precludes evaluation of phenotype in adult mice. Thus, pathologists are being increasingly called upon to familiarize themselves with fetal development and evaluate developmental defects. Fetal pathology is beyond the scope of this text, but the reader can access several excellent sources of information (see Kaufman 1995; Kaufman and Bard 1999; Rossant and Tam 2002; Ward et al. 2000). Embryonic/fetal viability is most often influenced by abnormalities in placentation, liver function, or cardiovascular function (including hematopoiesis). Particular attention should be paid to these factors. Depending upon genetic background, lethality can vary. Gene expression, and therefore circumvention of events such as embryonic lethality, can be controlled temporally and quantitatively by tissue-specific promoters with drug-regulated transcription systems and with cre/lox deletion, in which cre recombinase can be controlled with transcription techniques. Temporal and quantitative control of transgenes poses unique challenges to pathologists when evaluating phenotype.

In addition to predicted phenotypes, GEMs often manifest unique pathology that is not present in parental strains. Genetic constructs are usually inserted into the genome with a promoter to enhance expression, to target expression within a specific tissue, or to conditionally express the transgene, but promoters can affect phenotype as much as the gene of interest. Promoters are seldom totally tissue-specific and can impact upon other types of tissue. Conversely, overexpression of transgenes, regardless of their nature, can result in abnormalities in normal cell function. Tumors, particularly malignant tumors of mesenchyme, including hemangiosarcomas, lymphangiosarcomas, fibrosarcomas, rhabdomyosarcomas, osteosarcomas, histiocytic sarcomas, and anaplastic sarcomas, are frequent spontaneous lesions in transgenic mice that are relatively rare in parental strains of mice. Lymphoreticular tumors, which are quite common in parental strains of mice, reach epic proportions in GEMs. In some cases, relatively rare forms of lymphoma, such as marginal zone lymphomas, arise frequently in GEMs. Tumor phenotypes found in transgenic mice bearing *myc*, *ras*, and *neu* are distinctive and found only in mice with these transgenes. Many gene alterations have specifically targeted immune response genes, but others have unintentional effects upon immune response. When the immune responsiveness of the mouse is altered, opportunistic pathogens become an important factor in phenotype. Phenotypes have been known to disappear when mutant mice are rederived and rid of their adventitious pathogens.

Consequently, the pathologist must be cognizant of general mouse pathology, strain-related patterns of spontaneous pathology, infectious disease pathology, developmental pathology, comparative pathology (to validate the model), methodology used to create the mice, predicted outcomes of the gene alteration (including effects of the promoter), potential but unexpected outcomes of the gene alteration, and Mendelian genetics. The pathologist must also resist temptation to overemphasize a desired phenotype, underemphasize an undesired phenotype, or proselytize a phenotype as a model for human disease when it isn't. There is no better person to be the gatekeeper of reality in the world of functional genomics than the comparative pathologist.

ANATOMIC FEATURES

The laboratory mouse has several unique characteristics, and there are vast differences in normal anatomy, physiology, and behavior among different strains of mice, many of which represent abnormalities arising from homozygosity of recessive or mutant traits in inbred mice.

Integumentary System

The history of the laboratory mouse is steeped in selective breeding for variation in coat color and consistency, with many defined mutants. Hair growth occurs in cyclic waves, beginning cranially and progressing caudally. Examination of mouse skin mandates awareness of the growth cycle and location examined. Melanin pigment is restricted to the hair follicular epithelium and hair shaft, with minimal pigmentation of the interfollicular epidermis. Thus, newborn mice, regardless of their ultimate coat color, are uniformly pink until hair growth begins.

Hematology and Hematopoeitic System

Mouse hematology has been recently reviewed (Everds 2007). Strain-specific data and comparisons among inbred mouse strains are available through the Mouse Phenome Database. Recommended approaches to

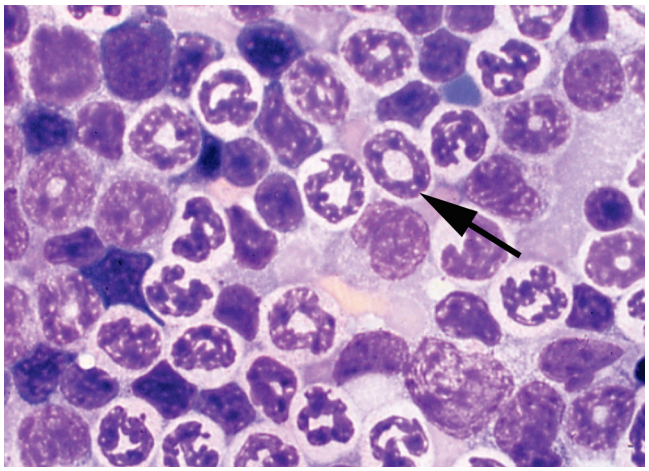

FIG. 1.1. *Ring-shaped nuclei (arrow) of myeloid progenitor cells in the bone marrow of a normal mouse.*

evaluation of GEMs with hematological phenotypes are also available (Car and Eng 2001). Mouse erythrocytes are small, with a high reticulocyte count, moderate polychromasia, and anisocytosis. Lymphocytes are the predominant circulating leukocyte and constitute approximately three-fourths of the total differential count. Mature male mice have significantly higher granulocyte counts than do female mice. Peripheral blood granulocytes tend to be hypersegmented, and band cells are rare, except when mice have chronic suppurative infections. Granulocytes in tissues and bone marrow often have ring-shaped nuclei (Fig. 1.1). Ring-shaped nuclei can be visualized as early as the progranulocyte stage in bone marrow, spleen, and liver, and only rarely can be found in peripheral blood. They also occur in cells of the monocytic lineage. Mice have circulating basophils, but they are extremely rare. Mice possess a very large platelet mass, due to high platelet numbers and relatively low mean volume, although some platelets can be as large as erythrocytes. The spleen is a major hematopoietic organ throughout life in the mouse, and hematopoiesis is found in the liver up to weaning age but may return in adults during disease states. Hepatic hematopoiesis can be misconstrued as inflammation. Hematopoiesis remains active in long bones throughout life.

Respiratory System

Cross sections of the nose reveal prominent vomeronasal organs, which are important in pheromone sensing and are frequent targets of viral attack. Virus-associated vomeronasal and olfactory rhinitis in neonatal mice can result in failure to suckle. Respiratory epithelium may contain eosinophilic secretory inclusions (hyalinosis), which are especially obvious in B6 and 129 mice. The lungs have a single left lobe and 4 right lobes. Cartilage surrounds only the extrapulmonary airways in mice, rats, and hamsters. Thus, primary bronchi are extrapulmonary. Respiratory bronchioles are short or nonexistent. Cardiac muscle surrounds major branches of pulmonary veins and should not be misconstrued as medial hypertrophy. Bronchus-associated lymphoid tissue is normally present only at the hilus of the lung, except in hamsters. Lymphoid accumulations are present on the visceral pleura of mice, within interlobar clefts. These are organized lymphoid structures that are contiguous with the underlying lung tissue and are similar to "milkspots" in the peritoneum. Although not a normal finding, focal intra-alveolar hemorrhage is a consistent agonal finding in lungs of mice, regardless of the means of euthanasia. As in other species, focal subpleural accumulation of alveolar macrophages (alveolar histiocytosis) is common (see Rat chapter 2, "alveolar histiocytosis").

Gastrointestinal System

Mice are coprophagic, with approximately one-third of their dietary intake being feces. Stomach contents will reflect this behavior. Incisive foramina, located posterior to the upper incisors, communicate between the roof of the mouth and the anterior nasal cavity. Incisors grow continuously, but cheek teeth are rooted. Mice have no deciduous teeth, and their incisors are pigmented due to deposition of iron beneath the enamel layer. One of several sexual dimorphisms in the mouse is found in the salivary glands. The submandibular salivary glands in sexually mature males are nearly twice the size as females and parotid salivary glands are also larger. Male submandibular glands have increased secretory granules in the cytoplasm of serous cells (Fig. 1.2). These glands undergo similar masculinization in pregnant and lactating females. The intestine is simple. Gut-associated lymphoid tissue (Peyer's patches) is present in both the

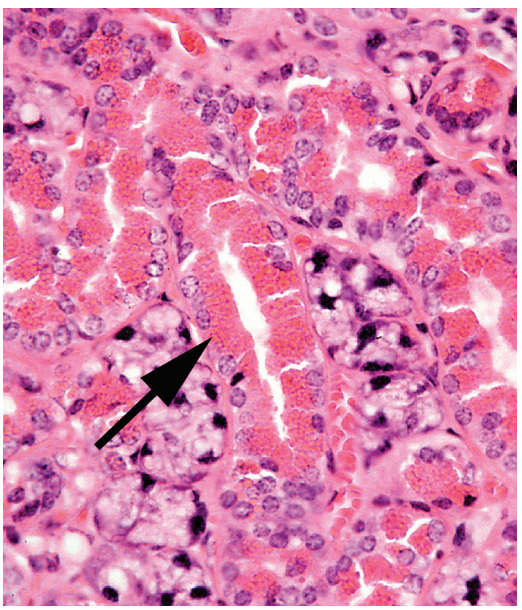

FIG. 1.2. *Submandibular (submaxillary) salivary gland from an adult male mouse. Note the prominent secretory granules (arrow) in the cytoplasm of epithelial cells.*

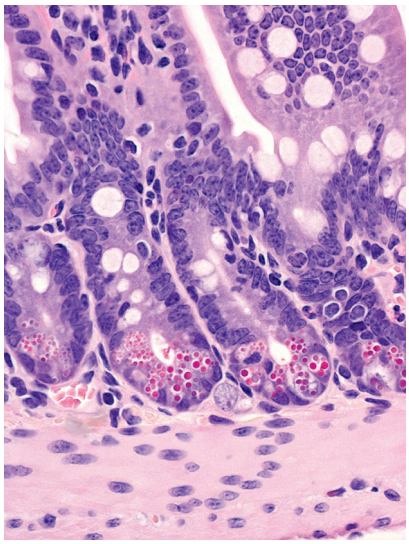

FIG. 1.3. *Ileal mucosa of a mouse illustrating the distinct cytoplasmic granules within Paneth cells at the base of the crypts.*

small and large intestine. Paneth cells occupy crypt bases in the small intestine. These specialized enterocytes have prominent eosinophilic cytoplasmic granules (Fig. 1.3), which are larger in mice than in other laboratory rodents. Pregnant and lactating mice have noticeably thickened bowel walls due to physiological mucosal hyperplasia. Mice have a very short (1–2 mm) rectum, which is the terminal portion of the large bowel that is not enveloped in serosa. Because of this feature, mice are prone to rectal prolapse, especially if they have colitis.

The intestine of neonatal mice has several unique features. Neonatal small intestinal enterocytes are vacuolated and may contain eosinophilic inclusions due to the presence of the apical–tubular system, which is involved in uptake of macromolecules (Fig. 1.4). It

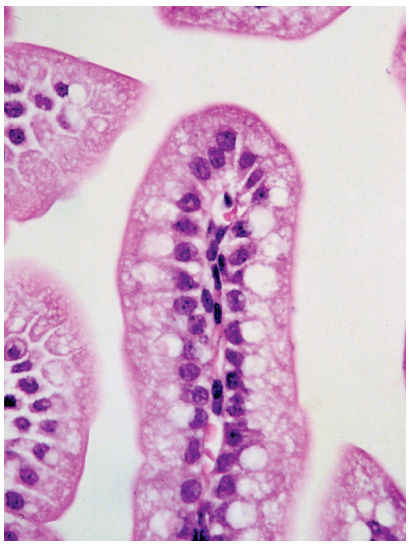

FIG. 1.4. *Enteric mucosa of a neonatal mouse, illustrating the vacuolated appearance of villus enterocytes.*

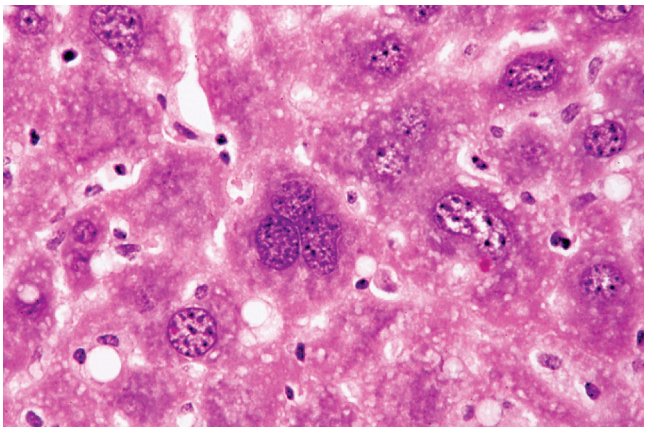

FIG. 1.5. *Polykarya and megalokarya, indicative of polyploidy, are commonly found in the liver and increase with age and disease states.*

disappears as the intestine undergoes maturation. The neonatal mouse bowel has very shallow crypts of Leiberkuhn populated with mitotically inactive stem cells and very long villi that are populated with terminally differentiated, absorptive epithelium. Intestinal cell turnover kinetics are slow in the neonate, making neonates highly vulnerable to acute cytolytic viruses. Turnover kinetics accelerate with acquisition of microflora and dietary stimuli.

The liver of mice has variable lobation. Polyploidy is common in mouse liver cells. Hepatocytes frequently display cytomegaly, anisokaryosis, polykarya, and karyomegaly (Fig. 1.5). Cytoplasmic invagination into the nucleus is frequent, giving the appearance of nuclear inclusion bodies (Fig. 1.6). Hematopoiesis normally occurs in the infant liver (Fig. 1.7) but wanes by weaning age, although islands of myelopoiesis or erythropoiesis can be found in hepatic sinusoids of older mice,

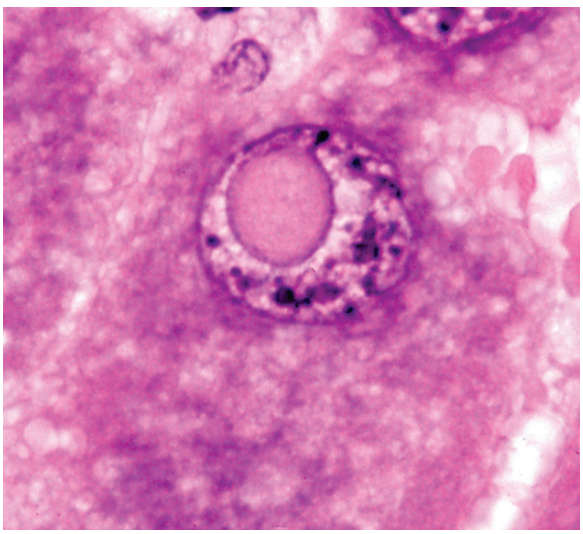

FIG. 1.6. *Cytoplasmic invagination into the nucleus of a hepatocyte, a common finding in rodents that has been misinterpreted as viral inclusions.*

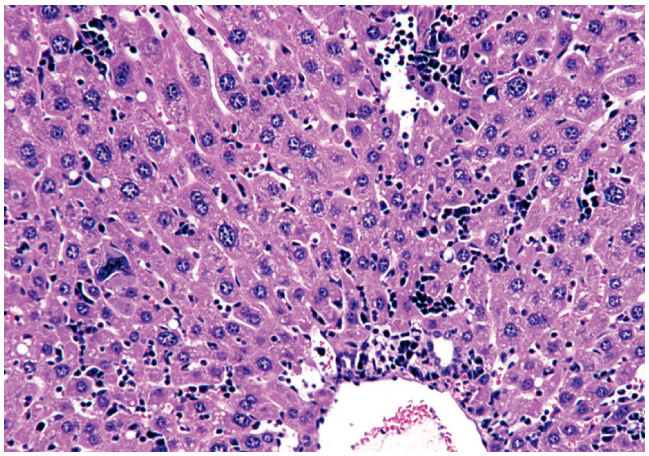

FIG. 1.7. *Liver from a newborn mouse. There are numerous hematopoietic cells in the sinusoidal regions.*

particularly in disease states (Fig. 1.8). Hepatocytes frequently contain cytoplasmic fat vacuoles. Some strains, such as BALB mice, normally have diffuse hepatocellular fatty change, resulting in grossly pallid livers, compared with the mahogany-colored livers of other mouse strains.

Genitourinary System

Female mice have a large clitoris, or genital papillus, with the urethral opening near its tip, which is located anterior to the vaginal orifice. Females that develop in utero between male fetuses are somewhat masculinized, reflected by an increased anogenital distance and behavior. Tissues of the adult uterine wall are normally infiltrated with eosinophils, which wax and wane cyclically and disappear during pregnancy. Eosinophils increase in number in response to semen. Mice have hemochorial placentation. Males have large redundant testes that readily retract into the abdominal cavity through open inguinal canals, particularly when the mice are picked up by the tail. Both sexes have well-developed preputial (or clitoral) glands, and males have

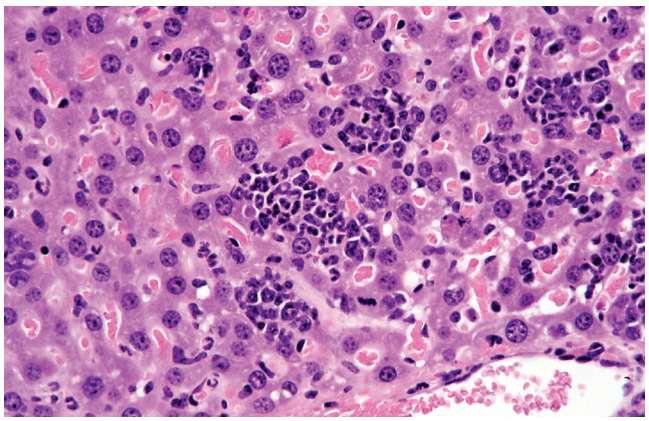

FIG. 1.8. *Liver from an adult mouse with suppurative pyelonephritis, illustrating marked hepatic myelopoiesis.*

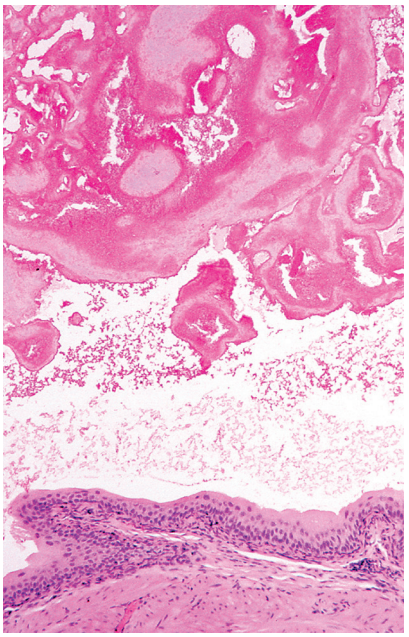

FIG. 1.9. *Copulatory plug in the urinary bladder of a male mouse. The presence of ejaculate coagulum is common in the urethra and bladder as an agonal finding, although antemortem ejaculation may result in urinary obstruction.*

conspicuous accessory sex glands, including large seminal vesicles, coagulating glands, and prostate. Ejaculation results in formation of a coagulum, or copulatory plug. This frequently occurs agonally. Coagulum can be found in urinary bladder or urethra as a normal incidental finding at necropsy (Fig. 1.9) and must not be misconstrued as a calculus or obstruction. However, copulatory plugs can and do cause obstructive uropathy. Sexual maturity in males results in several sexual dimorphic features, including larger kidneys, larger renal cortices, larger cells in proximal convoluted tubules, larger renal corpuscles, and cuboidal epithelium lining the parietal layer of Bowman's capsule, resembling tubular epithelium (Fig. 1.10). This is not absolute, since some glomeruli of male mice are surrounded by flat epithelium and some glomeruli of female mice are surrounded by cuboidal epithelium. Mice are endowed with relatively large numbers of glomeruli per unit area, compared with other species, such as the rat. Mice have a single, long renal papilla that extends into the upper ureter. Proteinuria is also normal in mice, with highest levels in sexually mature male mice. Major contributors to proteinuria in male mice are "mouse urinary proteins," which function as pheromones. In particular, MUP-1 is highly antigenic and a major cause of occupational allergies among animal handlers.

Endocrine System

The mouse adrenal gland has several notable features. The adrenals of male mice tend to be smaller and have less lipid than those of females. Accessory adrenals, either partial or complete, are very common in the

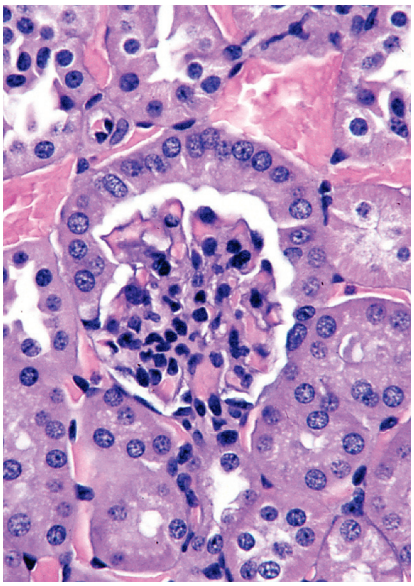

FIG. 1.10. *Renal cortex from an adult male mouse, illustrating the cuboidal epithelium lining the parietal surface of Bowman's capsule.*

adrenal capsule or surrounding connective tissue. The zona reticularis of the adrenal cortex is not discernible from the zona fasciculata. Proliferation of subcortical spindle cells, with displacement of the cortex, is common in mice of all ages (Fig. 1.11). The function of these cells is not known. A unique feature of the mouse adrenal is the X zone of the cortex, which surrounds the medulla. The X zone is composed of basophilic cells and appears in mice around 10 days of age. When males reach sexual maturity and females undergo their first pregnancy, the X zone disappears. The X zone disappears gradually in virgin females. During involution, the X zone undergoes marked vacuolation in females

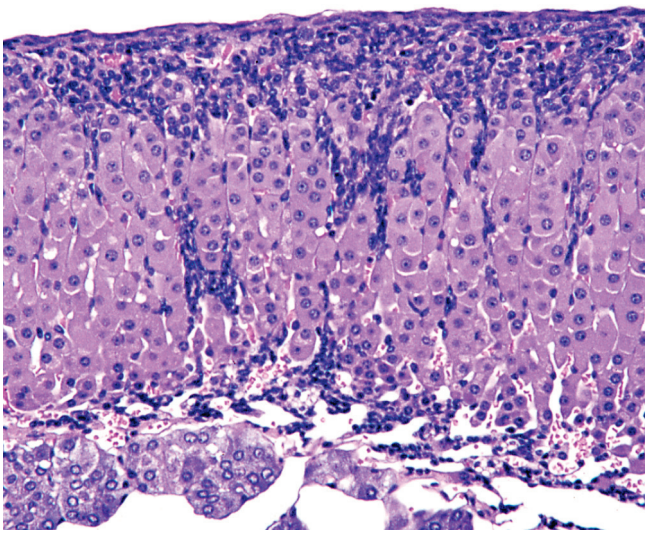

FIG. 1.11. *Subcapsular spindle cell proliferation in the adrenal gland of a normal adult mouse. This change is common, but its significance is not known.*

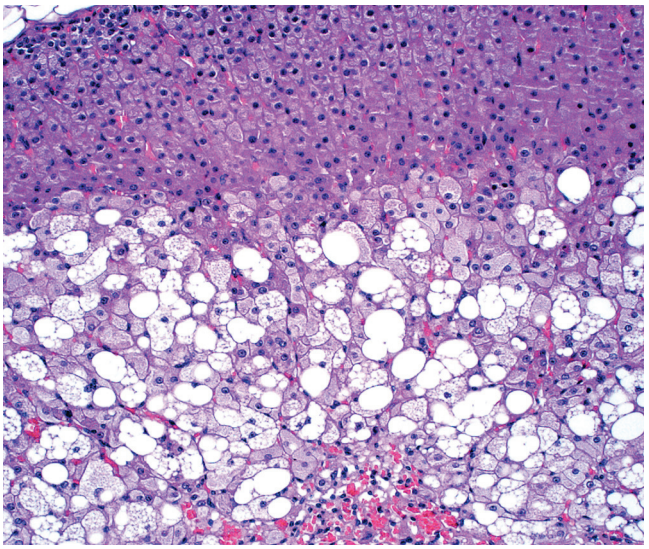

FIG. 1.12. *Vacuolating degeneration of the involuting X zone at the corticomedullary junction of the adrenal gland of an adult female mouse.*

(Fig. 1.12) but not in males. Residual cells accumulate ceroid. Pancreatic islets are highly variable in size, including giant islets that can be confused with hyperplasia or adenomas.

Skeletal System

Bones of mice, like those of rats and hamsters, do not have Haversian systems, and ossification of physeal plates with age is variable and incomplete, depending upon mouse genotype.

Lymphoid System

Rodents do not have tonsils, but have nasal-associated lymphoid tissue (NALT). Germinal centers are not well defined in lymph nodes. The thymus does not involute in adults. Hassall's corpuscles are indistinct. Islands of ectopic parathyroid tissue may be encountered in the septal or surface connective tissue of the thymus, and, conversely, thymic tissue may occur in thyroid and parathyroid glands. Epithelial-lined cysts are also common. The splenic red pulp is an active hematopoietic site throughout life (Fig. 1.13). During disease states and pregnancy, increased hematopoiesis can result in splenomegaly. Lymphocytes tend to accumulate around renal interlobular arteries, salivary gland ducts, urinary bladder submucosa, and other sites, increasing with age. These sites are often involved in generalized lymphoproliferative disorders. Melanosis of the splenic capsule and trabeculae is common in melanotic strains of mice (Fig. 1.14). This must be differentiated from iron (hemosiderin) pigment (Fig. 1.15), which tends to accumulate in the red pulp as mice age, particularly in multiparous females. Mast cells can be frequent in the spleen of some mouse strains, such as A strain mice.

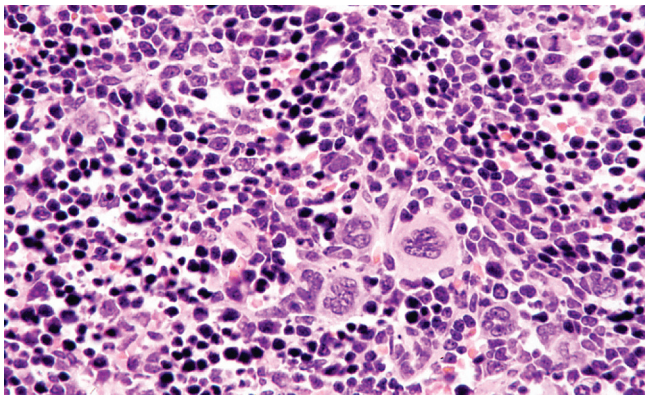

FIG. 1.13. *Spleen from an adult mouse, illustrating the large numbers of hematopoietic cells, including megakaryocytes, in the sinusoids, a common finding throughout life.*

Other Anatomic Features

The brain and spinal cord are larger in mature male mice compared to females. Melanosis occurs in the anteroventral meninges of the olfactory bulbs, optic nerves, parathyroid glands, heart valves, and spleens of melanotic mouse strains, such as B6 mice. Foci of cartilage or bone can be found within the base of the aorta. These foci are not an os cordis but rather occur within the wall of the aorta. Mice have 3 pectoral and 2 inguinal pairs of mammary glands, with mammary tissue enveloping much of the subcutis, including the neck. Mammary tissue can be found immediately adjacent to salivary glands, which is especially apparent during lactation. Nipple development is hormonally regulated in mice, and nipples are quite small in males. Mammary tissue of males totally involutes during development. Remarkably, virgin female mice can be induced to lactate by the presence of other females nursing litters. Mammary glands normally involute between pregnancies, but they do not involute in multiparous FVB mice, due to a tendency to develop hyperplasia of prolactin-producing cells and pituitary adenomas. Brown fat is prominent as a subcutaneous fat pad over the shoulders and is also present in the neck, axillae, and peritoneal tissue.

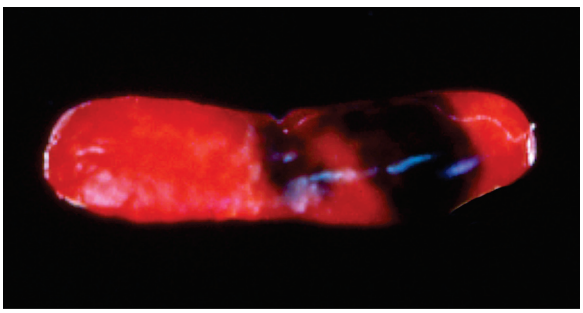

FIG. 1.14. *Splenic melanosis in a melantotic (C57BL) mouse. Note the patches of pigmented capsule.*

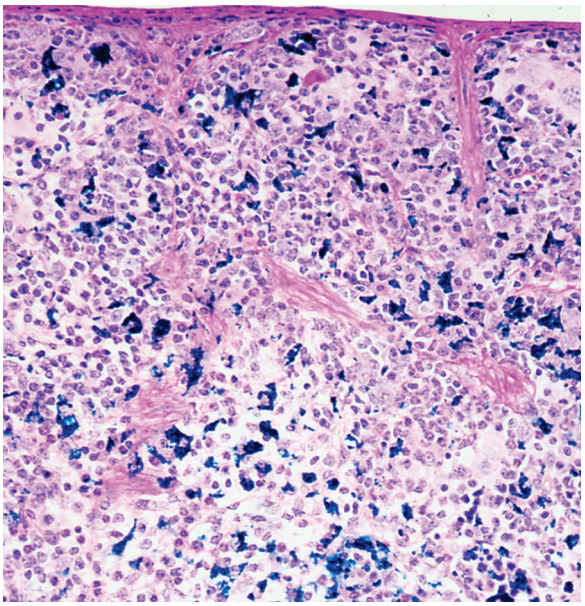

FIG. 1.15. *Iron pigment (hemosiderin) in the spleen of an adult female mouse (Perl's stain).*

Immunologic Idiosyncracies

Neonatal mice are globally immunodeficient. Different components of the innate and acquired immune response subsequently evolve at differing rates, depending upon genetic background. Although mice are generally immunocompetent at weaning, they are not fully so until 6–12 weeks of age. Neonates depend upon acquisition of maternal antibody to protect them during early life. Maternal IgG is transferred in utero through Fc yolk sac receptors, and postnatally through IgG receptors in the small intestine, which actively acquire immunoglobulin up to 2 weeks of age. Milk-borne IgA is also important in protecting suckling mice, but neither IgA nor IgM are absorbed. Passive immunity is a critical component in understanding the outcome of viral infections in mouse populations. Epizootic infections can be devastating in naïve populations of neonates, but once the infection becomes enzootic within a population, maternal antibody protects suckling mice during their period of age-related vulnerability. Maternal antibody generally persists in the serum of pups for about 6 weeks.

The immune response can vary considerably among different strains of mice. An often-cited feature is the Th1-Th2 polarized T-cell response, in which BALB/c mice tend to respond to antigenic stimuli with a Th2 skewed response and B6 mice with Th1 skewed responses. This is far from absolute, but there seems to be truth in the concept that B6 mice deal more efficiently with viral infections. B6, B10, SJL, and NOD mice have their own unique immunoglobulin isotype, IgG2c, in lieu of, but distinct from, IgG2a. IgG2c is not an allelic variant of IgG2a, since in these strains the IgG2a gene is

completely absent, and in IgG2a-positive strains, the IgG2c gene is absent. This may impact accurate measurements of humoral responses. The mouse genome possesses approximately 40 histocompatibility loci, and the major histocompatibility (MHC) loci are located on chromosome 17 within the MHC complex, known as the *H-2* complex. Each inbred strain of mouse has a defined *H-2* haplotype, or combinations of alleles, which are well-recognized determinants of strain-specific immune responses, including responses to infectious disease. Because of the inbred nature of laboratory mouse strains, *H-2* haplotype is a singularly important strain characteristic.

Various stressors, including dehydration, hypothermia, and acute infections, may result in massive corticosteroid-induced lymphocytic apoptosis. This is accompanied by generalized lymphoid depletion and transient nonspecific alterations of immune responsiveness. This is especially apparent in the thymus and is a frequent and rapid onset lesion in "water bottle accidents," when mice become hypothermic or dehydrated. Recently rederived and xenobiotic mice have lymphoid hypoplasia, accompanied by functional hyporesponsiveness.

Genetic engineering has given rise to many immunologic mutants of mice, and other naturally arising immune mutants have also been popularized, such as nude (T-cell-deficient), SCID (B- and T-cell-deficient), and beige (NK cell-deficient) mice. The preeminent immunodeficient mouse is the NSG mouse, discussed above. Immunodeficient mice must never be considered to be simply missing a single functional component of the immune system, since they typically have compensatorily activated innate and acquired immune responses compared to wild-type. Homozygous immunodeficient inbred mouse mutants that are progeny of the heterozygous (immunocompetent) parental matings or through embryo transfer into immunocompetent recipients can acquire functional immunoglobulin-secreting B cells from their immunocompetent dams. They can also acquire functional B cells postnatally through foster nursing. The chimeric cells may remain functional for at least several months.

Less obvious and often overlooked immunologic idiosyncrasies also exist among common inbred strains. All strains of adult male mice manifest a sexual dimorphism in which serum levels of both C4 and C5 are higher than in females, and male SJL mice have a significantly higher level of C5 compared to males of other strains. In addition, inadvertent consequences have arisen from inbreeding and selection for other characteristics. One such common defect is a 2 base pair gene deletion in the 5th component of complement (C5). This mutation results in C5 deficiency in many inbred strains of mice, including AKR, SWR, DBA/2J, A/J, A/HeJ, NOD, and RF, among others. SJL mice are NK cell-deficient. NOD mice have multiple immune defects (see above). Substrain divergence due to spontaneous acquisition of mutations can give rise to novel new substrain phenotypes, such as the LPS unresponsiveness of C3H/HeJ and C57BL/10ScN mice, which is attributed to a mutation of toll-like receptor 4 (TLR4). All strains of mice lack functional TLR10 due to genetic disruption by a retroviral insertion. CBA/CaN (CBA/N), but not other CBA mice, have an X-linked defect in humoral immunity, with impaired maturation of B cells, diminished immunoglobulin production, and impaired T-independent immune responses. Thus, knowledge of specific strain and substrain characteristics greatly improves the understanding of responses to experimental variables.

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INFECTIONS OF LABORATORY MICE: EFFECTS ON RESEARCH

Laboratory mice are host to a large spectrum of over 60 different infectious agents that may, under some circumstances, be pathogens. Many of these agents have been eliminated from contemporary mouse colonies but may re-emerge periodically. Declaring an infectious agent a pathogen in the laboratory mouse can be a challenge. Some agents produce no discernible pathology, even in

immunodeficient mice (e.g., astrovirus); some are opportunistic pathogens (e.g., *Pseudomonas*); and others (e.g., mouse hepatitis virus) can be overtly pathogenic in naïve neonatal mice or immunodeficient mice, and yet produce minimal or no signs when enzootic within a population or when infecting genetically resistant mice. These features create a challenge for educating the investigator about the significance of infectious agents in the mouse and convincing institutional officials of the need to provide core support for surveillance and diagnostic programs that ensure the health and welfare of research animals, as well as protecting the research investment. There are 3 major reasons for being concerned about infectious agents in the mouse: jeopardy of unique colonies, zoonotic risk, and effects on research. Effects on research are significant and varied, and there is growing documentation of infectious agents obscuring phenotypes in GEMs.

This text emphasizes all known naturally occurring infections of laboratory mice that have the potential for producing either lesions in mice or effects upon research, even those that have largely disappeared from contemporary mouse populations. This is because of the expanding use of immunologically deficient mice, burgeoning (and overcrowded) mouse populations, variable or inadequate microbial control practices, infestation of animal facilities by feral mice, and the re-emergence of rare infectious agents due to unrestricted traffic of GEMs among institutions. Microbial quality control is often a casualty in the face of financial austerity, imposed by declining National Institutes of Health budgets, rising husbandry costs, and increasingly onerous government and institutional regulations. All of these factors are contributing to the re-emergence of infectious disease among laboratory mice.

Disease expression is significantly influenced by age, genotype, immune status, and environment of the mouse. Genetic manipulation has introduced additional and often unexpected variables that may influence disease expression. Under most circumstances, even the most pathogenic murine viral agents cause minimal clinical disease. However, under select circumstances, the same agents can have devastating consequences. Genetically immunodeficient mice and infant mice less than 2 weeks of age that have not benefited from maternal immunity are highly susceptible to viral disease. Mouse strain genetic background, including *H-2* haplotype, is an important factor in host susceptibility, with growing nuances contributed by experimentally induced gene alterations. Different viruses, and different strains of virus, vary considerably in their contagiousness and virulence, which impacts sampling size for surveillance and recognition of disease. Housing methods, including ventilated cages and microisolator cages, complicate detection and significantly influence the contagion dynamics within a population. Infectious agents can be introduced to mouse colonies through feral mice, unrestricted traffic of personnel, biologic material, including transplantable tumors, ES cells, and iatrogenic introductions of mouse pathogens when used as models for human disease.

Investigation of host–agent epizootiology by the astute diagnostician must encompass all of these factors. Animals submitted for necropsy should be accompanied by thorough clinical history, including microbial surveillance data of the colony, accurate nomenclature, genetic background, and genetic manipulation. Mice must be carefully selected to provide maximal opportunity for diagnosis. Clinically ill animals or live cagemates of deceased or ill mice are optimal, since they would be most likely to have active infections or lesions. Diagnosis of infections in a rodent colony should not be solely dependent upon gross and microscopic pathology. A useful adjunct is serology, but this should never be used alone for diagnosis. Mice may be seronegative if actively infected with acutely cytolytic viruses, such as mouse hepatitis virus (MHV), and will be seropositive during or following recovery. Conversely, mice may be seropositive yet actively infected with a second strain of the same agent, as is the case with MHV. Young mice can be seropositive due to passively derived maternal antibody but not actively infected with the agent in question. Some virus infections, such as Sendai virus, induce immune-mediated disease. Thus, mice may not become clinically ill until a week or more into infection. Therefore, positive seroreactivity would be confirmatory in clinically ill mice infected with Sendai virus. These examples underscore that seroconversion to an agent does not imply a cause and effect relationship with disease, unless epizootiology, pathology, and serology are considered collectively. Finally, molecular methods of detection are increasing in use but must be accompanied by appropriate positive and negative controls, and positive results must always be confirmed by sequencing or other methods.

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DNA VIRAL INFECTIONS

Adenovirus Infections

Mice are host to 2 distinct adenoviruses, murine adenovirus-1 (MAdV-1) and murine adenovirus-2 (MAdV-2), which should be more accurately termed mouse adenovirus 1 and 2. MAdV-1 and MAV-2 can be differentiated from each other genetically, serologically, and by pathology. MAdV-1 and MAdV-2 differ significantly at the DNA level, with MAdV-2 having a distinctly larger genome. Adenoviruses are nonenveloped DNA viruses that replicate in the nucleus. Infection results in pathognomonic intranuclear inclusions.

Epizootiology and Pathogenesis

MAdV-1 (previously FL) was initially discovered as a contaminating cytopathic agent during attempts to establish Friend leukemia (FL) virus in tissue culture. Infection is transmitted by direct contact through urine, feces, and nasal secretions. Serologic surveys have indicated that MAdV-1 was at one time common in laboratory mouse colonies, but it is now rare or nonexistent in laboratory mice from North America and Europe. Naturally occurring clinical disease or lesions due to MAdV-1 have not been described, but experimental intraperitoneal inoculation of neonatal, suckling, and immunodeficient mice with MAdV-1 results in viremia and a fatal, multisystemic infection within 10 days. MAdV-1 infects cells of the monocyte–macrophage lineage, microvascular endothelial cells, respiratory epithelium, adrenal cortical cells, and renal distal tubular cells. Experimental inoculation of weanling or adult mice results in multisystemic infection with leukocyte-associated viremia and prolonged viruria. There are marked mouse strain differences in susceptibility to experimental infection. Mice less than 3 weeks of age are universally susceptible to experimental disease, and adult C57BL/6, DBA/2, SJL, SWR, and outbred CD-1 mice tend to be susceptible to lethal experimental disease, whereas BALB/c, C3H/HeJ, and most other inbred strains tested are disease resistant. Susceptible strains develop hemorrhagic encephalitis, which does not occur in resistant strains. The nature of infection of immunodeficient strains of mice depends upon strain genetic background. BALB-*scid* and BALB-*scid*/*beige* mice develop fatal disseminated infection with focal hemorrhagic enteritis and microvesicular fatty change in the liver, consistent with Reye's-like syndrome, but no neurologic involvement. B6-*Rag1* mice develop disseminated disease with hemorrhagic encephalomyelitis. Athymic C3H/HeN-*nude* mice develop a progressive wasting disease with disseminated infection and duodenal hemorrhage, but no central nervous system involvement. Thus, genetic background is a major determinant of susceptibility to lesions in the central nervous system. Lymphoid B cells are critical for controlling disseminated infection, and T cells are required for recovery from infection but also contribute to pathology.

MAdV-2 (K87) was initially isolated from feces of an otherwise healthy mouse. In contrast to MAdV-1, MAdV-2 is principally enterotropic, regardless of route of inoculation, mouse strain, or immunosufficiency. Following oral inoculation of mice less than 4 weeks of age, MAdV-2 is excreted in feces for 3 or more weeks with peak infection between 7 and 14 days. Immunocompetent mice apparently recover. Seroconversion of rats to MAdV-2 has been noted, but they are not susceptible to experimental inoculation with the mouse virus, suggesting that they are host to a related, but different, adenovirus (see Rat Chapter 2, Rat Adenovirus Infection).

Pathology

Natural infection of immmunocompetent adult mice with MAdV-1 is typically subclinical, but the growing number and use of immunodeficient strains of mice warrant awareness of experimental findings. Mice experimentally inoculated with MAdV-1 develop runting, dehydration, thymic involution, and grossly evident foci of necrosis in liver, spleen, and other organs. Intranuclear inclusions can be found within foci of necrosis and hemorrhage in multiple organs, including brown fat, myocardium, cardiac valves, adrenal gland (Fig. 1.16), spleen, brain, pancreas, liver, intestine,

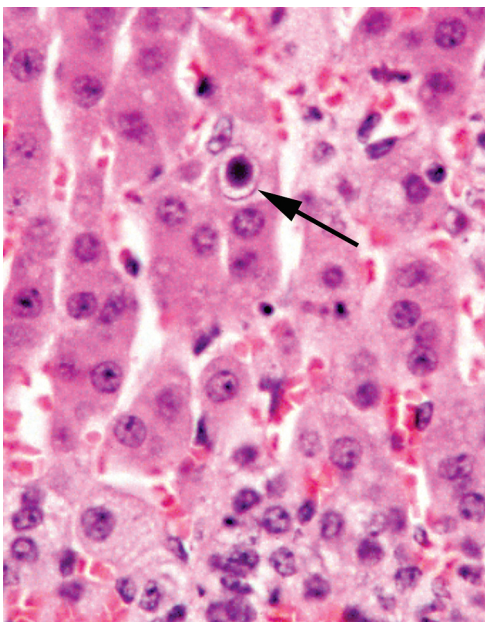

FIG. 1.16. *Adrenal cortex from a mouse experimentally infected with mouse adenovirus MAdV-1. A single intranuclear inclusion (arrow) is present in the cortical epithelium.*

salivary glands, and renal distal tubular epithelium. Peribronchiolar and pulmonary interstitial infiltration with mononuclear leukocytes have been found in adult B6 mice following intranasal inoculation. Focal hemorrhagic enteritis has also been noted in some genotypes of mice following experimental inoculation. Gastrointestinal tracts can be empty, with segmental inflammation and hemorrhage in the distal duodenum and jejunum. Inclusions in intestinal lesions tend to be difficult to visualize. Hemorrhagic foci may occur throughout the central nervous system but especially in the white matter of susceptible mouse strains. Endothelial cell necrosis is prominent in these foci, but inclusions are rare except in Purkinje cells. Central nervous system lesions can be clinically manifest as rigid tails, hypermetria, paraphimosis, ataxia, and urinary bladder distention.

Clinical signs are usually absent in MAdV-2 infected mice, but runting of suckling mice has been observed in natural infections. Naturally infected athymic nude mice are clinically normal. Gross lesions of MAdV-2 infection are not evident, except that juvenile mice may be bloated and runted. Mice naturally or experimentally infected with MAdV-2 develop intranuclear inclusions in mucosal epithelial cells of the small intestine, especially in the distal segments, and the cecum. Inclusions are most plentiful in infant mice but can also be found in smaller numbers in the mucosa of adult mice, especially dams that are suckling infected infants. Similar inclusions have been noted in adult nude mice without other detectable lesions. Typically, inclusion-bearing nuclei are located in the apical portions of cells, rather than in their normal basal location (Fig. 1.17).

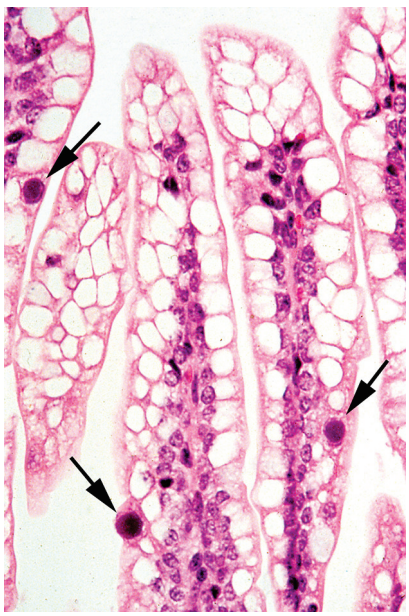

FIG. 1.17. *Small intestine from a juvenile mouse infected with mouse adenovirus MAdV-2. Note the distinct intranuclear inclusion bodies (arrows) in a few enterocytes lining the villi.*

Diagnosis

Serology is the most effective means of screening mouse populations for MAdV infection. These agents partially cross-react serologically, depending on method, in a one-way relationship with antiserum to MAdV-2 cross-reacting with MAdV-1, but not conversely. Since MAdV-1 is quite rare in laboratory mice, MAdV-2 antigen should be used. Differential diagnoses for MAdV-1 include multisystemic infections that produce intranuclear inclusions, such as polyoma virus and cytomegalovirus. Intestinal epithelial MAdV-2 inclusions are pathognomonic, but not always apparent. They are not induced by any other known agent, although K virus can form inclusions in intestinal endothelial cells. Inclusions in the apical region of enterocytes must be differentiated from mitotic cells and intraepithelial lymphocytes. Success at finding MAdV-2 inclusions is maximized in infant mice. MAdV strain-specific PCR can also be used.

Herpesvirus Infections

Mice are host to two members of the family Herpesviridae, subfamily Betaherpesvirinae and genus *Muromegalovirus*, including mouse cytomegalovirus (MCMV) and mouse thymic virus (MTV). Neither is common among contemporary laboratory mouse populations, but may contaminate archival biological products.

Mouse Cytomegalovirus Infection

MCMV is a mouse-specific virus that was originally isolated by M.G. Smith from salivary glands of naturally infected laboratory mice. Cytomegaloviruses replicate in the nucleus and cause cytomegalic inclusion disease, characterized by enlarged cells bearing both intranuclear and intracytoplasmic inclusions, particularly in salivary glands. MCMV has been studied extensively as an animal model of human CMV (HCMV) infection, but significant biological differences exist between MCMV and HCMV. Most laboratory studies have utilized the original Smith strain of MCMV, or derivatives thereof, and may not accurately reflect the natural biology of other MCMV strains. Nevertheless, the intense scrutiny of MCMV as a prototype model system has shed considerable light on its pathogenesis.

Epizootiology and Pathogenesis

Wild mice are commonly infected with MCMV. There are multiple genetically diverse MCMV strains within wild mouse populations, and mixed infections of single mice are common. Laboratory studies have found that infection-induced immunity to one strain does not preclude infection with a second strain. MCMV is transmitted oronasally by direct contact and is excreted in saliva, tears, urine, and semen. Experimental infection is significantly influenced by virus strain, dose, route of inoculation, and host factors (age, genotype). Neonates of all mouse strains are universally susceptible to severe

disease, and resistance to lethal disease evolves after weaning and increases until about 8 weeks of age. Genetically resistant mouse strains include B6, B10, CBA, and C3H mice, and susceptible strains include BALB/c and A strain mice. Resistance is associated with *H-2k* haplotype, but non-*H-2* associated factors also exist, including a resistance factor that is linked to loci on chromosome 6 within the natural killer (NK) cell complex. This region encodes a receptor expressed on NK cells that binds to a glycoprotein of MCMV, but MCMV isolates from wild mice have naturally occurring mutations that fail to activate NK cells through this receptor. NK cells interact with MCMV in other ways that are linked to the *H-2k* haplotype.

Following experimental inoculation of infant mice, viremia and multisystemic dissemination occur within 1 week, with infection of lung, heart, liver, spleen, salivary glands, and gonads. Macrophages are major targets of the virus, and blood monocytes are important for the viremic phase of infection. Following dissemination, virus is rapidly cleared from tissues, except from salivary gland. Infection of NK-deficient beige mice, or mice depleted of NK cells, significantly prevents virus clearance. Adaptive immune responses, including CD4 and CD8 cells, are also important in clearing infection from most sites. Athymic or SCID mice fail to control active infection, but B-cell-deficient mice can recover from acute infection. Curiously, despite functional innate and acquired immune responses in fully immunocompetent mice, including NK, CD4, CD8, and B cells, MCMV continues to persist and replicate in salivary gland tissues. A number of MCMV genes function to control the innate and acquired immune responses, in addition to determining cell tropism and inhibiting apoptosis. Most of these genes are not essential for virus replication in vitro, and therefore provide selective advantages for virus persistence in vivo. An important feature of MCMV (and other herpesviruses) is latent infection, in which virus persists in a nonreplicative state, but can be reactivated by immunosuppression or stress. Based upon tissue explanting and PCR, latency of MCMV has been documented in various organs, including salivary glands, lung, spleen, liver, heart, kidney, adrenal glands, and myeloid cells. This state of latency can persist for the life of the mouse.

Unlike HCMV, MCMV does not readily cross the placenta, and in utero transmission does not usually take place in naturally and experimentally infected mice. Infection of pregnant mice may cause fetal death and resorption, delayed birth, and runted pups, but these are nonspecific events. Nevertheless, latently infected dams have been documented to transmit low-level or latent infection to fetuses in utero. MCMV infects cells in the epididymis, seminal vesicles, testes, including Leydig cells and spermatozoa, as well as ovarian stromal cells. Experimental transmission by artificial insemination has been reported. Thus, sexual transmission is likely.

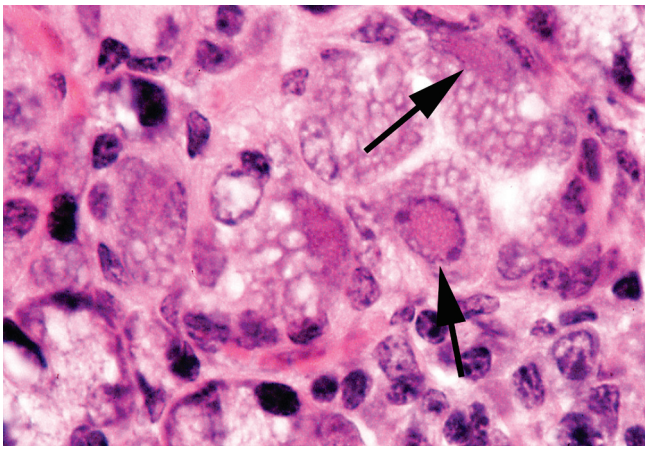

FIG. 1.18. *Submaxillary salivary gland from a wild mouse infected with mouse cytomegalovirus. Intranuclear inclusions (arrows) are present in acinar epithelial cells.*

Pathology

Overt disease and disseminated lesions do not usually occur in naturally infected mice. The most frequently encountered lesions occur in the submandibular salivary glands and, rarely, in the parotid glands. Eosinophilic intranuclear and intracytoplasmic inclusions are present in acinar epithelial cells with cytomegaly (Fig. 1.18) and lymphoplasmacytic infiltration of interstitium. During the acute disseminated phase in experimentally inoculated infant mice or T-cell-deficient mice, focal necrosis, cytomegaly, inclusions, and inflammation occur in many tissues, including salivary glands, lacrimal glands, brain, liver, spleen, thymus, lymph nodes, peritoneum, lung, skin, kidney, bowel, pancreas, adrenal, skeletal and cardiac muscle, cartilage, and brown fat. Diffuse interstitial pneumonitis has been described in BALB/c mice that were immunosuppressed by a variety of methods, and athymic mice develop progressive multifocal nodular pulmonary inflammation. Athymic mice also develop progressive destruction of adrenals. Arteritis of the pulmonary artery and aorta (at the base of the heart) has been documented during experimental MCMV infection in B6 and BALB/c mice, but no virus was confirmed within the lesions. A single case of naturally occurring MCMV disseminated infection has been reported in an aged laboratory mouse. MCMV has been shown to have a synergistic effect with *Pseudomonas aeruginosa*.

Diagnosis

Lesions in salivary glands are typical of cytomegalovirus, but are not always present in infected animals. Serology is the method of choice for colony surveillance, but detection of infection in immunodeficient mice that do not seroconvert must be accomplished through nucleic acid detection, including in situ hybridization and PCR. These methods can also be used to detect latent infection. Differential diagnosis for sialoadenitis with inclusion bodies must include polyoma virus. Other

viruses that infect salivary glands include reovirus 3, mouse thymic virus, and mammary tumor virus, but these viruses do not induce inclusions.

Mouse Thymic Virus Infection

Detailed information about mouse thymic virus (MTV) is generally lacking, since in vitro methods of propagation have not been identified and little experimental work has been performed. Virtually nothing is known about its genome. Synonyms include thymic necrosis virus, thymic agent, and mouse T-lymphotropic virus. The prevalence of MTV in laboratory mouse populations is extremely rare, but it is very common among wild mice. MTV can be a contaminant of MCMV stocks prepared from salivary glands. Because of its lymphocytotropism, MTV infection may result in significantly altered immune responses.

Epizootiology and Pathogenesis

MTV was first discovered when inoculated newborn Swiss mice developed thymic necrosis following serial passage of mammary tumor homogenates. This feature of MTV has been emphasized in subsequent studies, but MTV infects salivary glands as its primary target. Outcome of experimental infection is strikingly age dependent and also influenced by mouse genotype. Intraperitoneal as well as oronasal inoculation of newborn mice results in acute thymic necrosis, which is visible grossly as diminished thymic mass, within 1–2 weeks. Subsequently, the thymus returns to normal, but mice remain persistently infected. Age-related susceptibility to thymic necrosis decreases progressively until 6 days of age, at which point mice are no longer susceptible to thymic necrosis. Although largely an experimental phenomenon, thymic necrosis has been encountered in infant mice from naturally infected mouse colonies. CD4+8+ and CD4+8– T cells are selectively targeted by MTV, although virus replication also occurs in thymic epithelial cells and macrophages. Newborn mice develop viremia, with MTV detectable in multiple organs. Mice of all ages develop infection of salivary glands, with persistent virus shedding in saliva for several months or more. Athymic mice, which lack the T-cell substrate for virus replication, tend to shed virus less consistently. The mode of MTV transmission is presumed to be primarily through the saliva, and MTV is readily transmitted by direct contact. MTV has also been isolated from mammary tissue of an infected mother and from mammary tumor extracts, suggesting another possible route of transmission. Vertical (in utero) transmission has not been documented.

Pathology

MTV infection of infant mice results in the formation of intranuclear inclusion bodies and necrosis of thymocytes and, to a lesser extent, cells in lymph nodes and spleens. During recovery, there is granuloma formation. Lesions in salivary glands have not been noted. BALB/c and A strain mice, but not B6, C3H, or DBA/2 mice, when inoculated as neonates develop gastritis. Mice of other strains develop oophoritis and antibodies to thyroglobulin. These phenomena are believed to be autoimmune in origin, via nonspecific activation and expansion of self-reactive T cells, and not related to MTV in these tissues.

Diagnosis

Seroconversion can be detected using infected salivary tissue as antigen. Mice infected as neonates may not seroconvert, probably due to immune tolerance. The mouse antibody production (MAP) test can be useful, but PCR is now available for testing mouse tissue and biologic products. Differential diagnoses include coronavirus or stress, which may cause thymic necrosis, but not inclusions. A bioassay has been used to detect MTV, in which inoculated infant mice develop thymic necrosis.

Parvovirus Infections

Laboratory mice are subject to infection with two different autonomously replicating types of viruses in the family Parvoviridae: minute virus of mice (MVM) and mouse parvovirus (MPV). The official and generally unaccepted name for MVM is mice minute virus (MMV), which will not be used in this text. MVM and MPV, including dual infections, are among the most prevalent viruses in contemporary laboratory mouse populations. MPV strains represent the predominant type (75%) in parvovirus-positive populations. Clinical disease is seldom present in immunocompetent mice, but these viruses have significant immunomodulatory effects, and they are remarkably refractory to effective eradication from contaminated mouse colonies.

MVM and MPV share considerable homology among genes that encode 2 antigenically cross-reactive (among all mouse parvoviruses) nonstructural proteins, NS1 and NS2, but display variation among genes that encode structural capsid proteins, VP1, VP2, and VP3. VP2, in particular, contributes to significant antigenic and biologic differences among mouse parvoviruses. Sequence analysis, differential PCR, and restriction fragment length polymorphism analysis have lent clarity to the rodent parvovirus interrelationships. The MVM group contains MVMp, MVMi, MVMm, and MVMc, and the MPV group contains a cluster of closely related MPV-1a, MPV-1b, MPV-1c, a somewhat disparate cluster containing MPV-2, and another cluster containing MPV-3 and a closely related hamster parvovirus that is closely related to MPV-3 and likely to be of mouse origin. The mouse parvoviruses are distinctly different from the parvoviruses of rats. More isolates and strains are likely to be discovered, and therefore it is most expedient to discuss the two major phylogenetic groups.

Epizootiology and Pathogenesis

Mouse parvoviruses are transmitted through feces and urine by oronasal exposure with a slow rate of cage-to-cage spread. In general, parvoviruses of all host species are dependent upon the S phase of the cell cycle for virus replication, and therefore induce cytolytic disease only in dividing tissues (including lymphoid tissues undergoing antigenic stimulation). However, virus replication, and therefore patterns of disease, is limited to certain cell types that bear the appropriate viral receptors. For example, many parvoviruses replicate in intestinal crypt epithelium, but rodent parvoviruses do not target that cell population, and therefore do not induce intestinal disease.

Following oral inoculation, mouse parvoviruses initially replicate in intraepithelial lymphocytes, lamina propria, and endothelium of the small intestine, and then disseminate to multiple organs, including kidney, intestine, lymphoid tissues, liver, and, to a much lesser extent, lung, with tropism for endothelial cells, hematopoietic cells, and lymphoreticular cells. Viremic dissemination is likely related to the high degree of lymphocytotropism of these viruses, but MVM viremia is also erythrocyte associated. Both types of virus target small intestine and lymphoid tissue, and MVM also replicates in kidney. MVM infection of both infant and adult immunocompetent mice is limited in duration, with recovery. In contrast, MPV infection is typically persistent following infection of mice of all ages, but juvenile mice may transmit virus more efficiently. Neonatal mice are protected from infection by maternal antibody in enzootically infected colonies. Mice resist reinfection with the homotypic virus, but are fully susceptible to infection with the heterotypic serotype, thus explaining the naturally high frequency of dual MVM and MPV infections.

Pathology

Natural infection of immunocompetent mice is clinically silent, regardless of age or strain of mice. Experimental infection of neonatal BALB/c, SWR, SJL, CBA, and C3H mice with MVMi has been shown to cause mortality due to hemorrhage, hematopoietic involution, and renal papillary infarction. DBA/2 neonates also developed intestinal hemorrhage and more rapid hepatic hematopoietic involution, whereas B6 neonates are resistant to vascular and hematopoietic diseases. MVM is more pathogenic for hematopoietic tissues than MPV. Infection of neonatal BALB/c mice with MVMi revealed replication and a significant decrease in bone marrow and splenic cellularity, with depressed myelopoiesis. MVM infection of SCID and neonatal mice has been found to induce lethal leukopenia, due to virus replication in primordial hematopoietic cells, with severe depletion of granulomacrophagic cells and compensatory erythropoiesis in bone marrow. Natural MVM-related disease has been observed in NOD.Cg-*H2*^{H4}-*Igh-6* null mice with leukopenia and anemia. Intranuclear inclusions were present in mononuclear cells of spleens (Fig. 1.19) and bone marrow. Experimental exposure of infant mice with MVM, including intranasal inoculation and contact exposure, results in virus replication in the cells of the subventricular zone, subependymal zone of the olfactory bulb, and the dentate gyrus of the hippocampus. These are the 3 main germinal centers in postpartum neurogenesis of the mouse. MVM also targets the outer granular layer of the cerebellum, with cytolysis of cells that have migrated to the internal granular layer, resulting in granuloprival cerebellar hypoplasia. In experimentally infected pregnant mice, MVM replication may occur in various tissues, including placenta and fetus, without histological evidence of lesions.

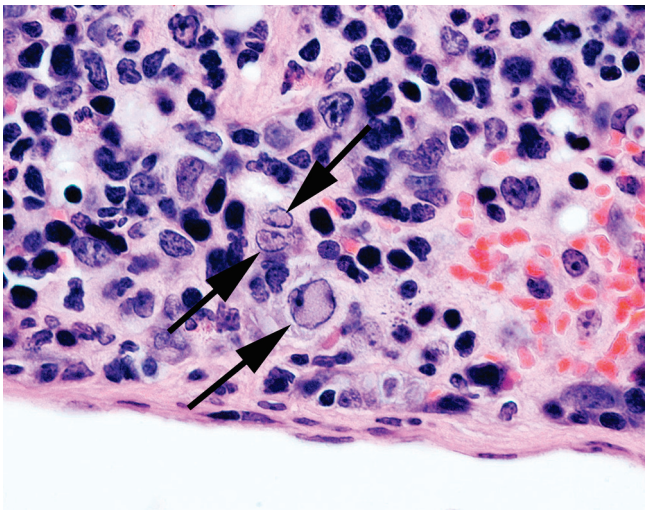

FIG. 1.19. *Intranuclear inclusions (arrows) in mononuclear cells of the spleen in an immunodeficient mouse naturally infected with minute virus of mice (MVM). (Source: Franklin 2006. Reproduced with permission from Oxford University Press.)*

Diagnosis

MVM and MPV infection is usually diagnosed by seroconversion, but colony surveillance can be challenging due to the inefficient cage-to-cage transmission of these viruses, thereby requiring large sample sizes for surveillance. Indeed, the inefficient cage-to-cage transmission has made it possible to test and cull as a means of eliminating infection from a population. Furthermore, experimental inoculation of adult ICR, BALB/c, C3H/HeN, C57BL/6, and DBA/2 mice with different doses of MPV has revealed marked differences in serologic response, with antibody detection in all C3H mice, some but not all ICR, BALB, and DBA mice, and none of the B6 mice, except when inoculated with very high doses of virus. ELISAs utilize the extensive cross-reactive recombinant NS1 antigen to detect antibody to both MVM and MPV, and recombinant VP2 antigen or empty capsids can be used to differentiate antibodies between

MVM and MPV. Caution is advised, since antibodies to new isolates or strains that are more distantly related to MVM or MPV may not be detected. PCR primers for detection of conserved and group-specific regions of the genome are available for detection of virus in tissues and feces. The mesenteric lymph node is an optimal tissue for PCR-based detection of mouse parvoviruses. A comparison of sensitivity of testing procedures resulted in the following percentage of positives: mesenteric lymph node PCR (93%), serologic immunofluorescence assay (68%), direct fecal PCR (10%), and cage fecal PCR (5%). The MAP test and virus isolation, including tissue explant cultures, are more labor-intensive methods that can also be utilized.

Papillomavirus Infections

A papillomavirus (MusPV) was discovered in an inbred NMRI-*nude* mouse colony, in which the mice developed florid papillomas at the mucocutaneous junctions of their nose and mouth. The agent induced papillomas in T-cell-deficient (athymic nude and SCID) strains of mice. MusPV is infectious, but not oncogenic, in a variety of immunocompetent strains of mice, which display varying degrees of susceptibility. MusPV is genetically closely related to the rat papillomavirus virus. Another *M. musculus* papillomavirus has since been reported from normal ear skin tissue of wild mice in Europe. Although these viruses are rare in laboratory mouse populations, they are likely to attain popularity as research models, with subsequent iatrogenic introduction to laboratory mouse populations.

Polyomavirus Infections

Mice are hosts to two genetically distinct polyomaviruses: polyoma virus (PyV) and K virus. They belong to the family Polyomaviridae, which contains a number of related viruses (macaque SV-40 virus, human BK and JC viruses, hamster polyomavirus, rat polyomavirus, and rabbit kidney vaculolating virus). These viruses once belonged to the family Papovaviridae, but that family has been disbanded and split into Polyomaviridae and Papillomaviridae. PyV encodes a middle T antigen that is important in oncogenesis. The K virus genome is similar to that of PyV, but lacks the middle T antigen of PyV. Both viruses are rare or nonexistent in contemporary laboratory mouse populations, but PyV continues to be used as an experimental oncogenic virus that on occasion results in iatrogenic introductions to susceptible mouse colonies.

Polyoma Virus Infection

PyV was originally termed the "Stewart-Eddy (SE) polyoma virus" and the "parotid tumor virus." The virus has been extensively studied as an oncogenic virus that induces many (poly) types of tumor (-oma). The name is well deserved, as tumors arise from more than a dozen different cell types. Under experimental conditions, it is oncogenic in several different species. The oncogenic activity for which this virus is so well known is largely an experimental phenomenon, requiring parenteral inoculation of genetically susceptible strains of mice within the first 24 hours of life with high titers of selected isolates virus with high oncogenic activity. The relevance of PyV to the laboratory mouse has risen with the use of PyV middle T (PyV-MT) gene as a component of transgenic constructs. PyV is known to contaminate transplantable tumors and cell lines, which in turn have served as inadvertent sources of contamination of mouse stocks.

Epizootiology and Pathogenesis

PyV was initially discovered by Ludwig Gross, when newborn mice unexpectedly developed salivary gland tumors following inoculation with filtered extracts of mouse leukemia tissue. PyV is an environmentally stable virus that is shed primarily in saliva, urine, and feces. Infection is most efficiently acquired intranasally. Infection of a mouse population requires a continuous source of exposure, which is provided by repeatedly utilized nesting sites of wild mice. The virus fails to survive under laboratory mouse husbandry conditions and is, therefore, quite rare in contemporary mouse colonies. Oronasal inoculation of neonatal mice results in virus replication in the nasal mucosa, submaxillary salivary glands, and lungs, followed by viremic dissemination to multiple organs, including kidneys. Mortality can be high at this stage. By day 12, the virus is cleared by the host immune response from most sites but persists in the lung and especially kidney for months, where virus replicates in renal tubular epithelial cells. Infection of older mice is more rapidly cleared, with inefficient virus excretion for shorter periods. Transplacental transmission does not seem to occur naturally, but virus can be reactivated in the kidneys of adult mice during pregnancy if they were infected as neonates. Since PyV is a widely used experimental virus, iatrogenic contamination of laboratory mice can take place, but consequences are limited for the reasons just cited. Thus, under natural conditions, maternal antibody from immune dams, coupled with the low level of environmental contamination in a laboratory mouse facility, precludes successful infection of neonatal mice and attenuates survival of the virus in the population.

The oncogenic characteristics of PyV have been extensively studied and are herein reviewed because pathologists may encounter mice that are either experimentally infected with the virus, inadvertently exposed to the virus as immunodeficient strains, or derived from PyV transgenic constructs. Not all strains or isolates of the virus are oncogenic, including many "wild-type" isolates. Virus strains that produce "large plaques" in cell culture can induce tumors in 100% of susceptible mice, whereas other strains, which produce "small plaques," induce few or no tumors. If genetically susceptible

mice are experimentally inoculated parenterally with oncogenic virus at less than 24 hours of age, microscopic foci of cellular transformation arise in multiple tissues. Most of these foci remain microscopic, but others grow rapidly into large tumors within 3 months. Tumors arising in mice inoculated with less oncogenic strains may not arise until 6–12 months, and the tumors that arise are usually mesenchymal, rather than epithelial in origin.

The genetic basis of susceptibility to PyV oncogenesis has been extensively studied in more than 40 inbred strains and F1 hybrids, with susceptibility ranging from 100% to complete resistance, with many intermediate phenotypes. Resistance can be determined by both immunological and nonimmunological factors. For example, C57BL mice are highly resistant due to effective antiviral and antitumor immunity, which can be abrogated by neonatal thymectomy, irradiation, or immunosuppression. C57BR mice are susceptible to infection as neonates but do not develop tumors. Other strains of mice are resistant to tumor induction, even when immunosuppressed. Susceptibility to tumor induction has been linked to *H-2k* haplotypes. C3H/BiDa (*H-2k*) are fully susceptible, whereas DBA/2 and BALB/c mice (*H-2d*) are resistant. In addition, susceptibility is conferred in many mouse strains by an endogenous mouse mammary tumor provirus, *Mtv-7*. *Mtv-7* encodes a superantigen (Sag) that, when expressed, results in deletion of Vß6+ T cells. This abrogates the ability of these mice to mount an effective antitumor cytotoxic T-cell response. Another, non-Sag mechanism for susceptibility has recently been discovered in wild-derived inbred mice.

The biological characteristics of PyV are uniquely suited for disseminated, polytropic infection and neoplasia. The virus protein VP1 nonselectively binds universally to sialic acid of cell surfaces, contributing to polytropism. The virus also possesses multivalent enhancer regions that enable it to be transcribed and replicate in many cell types. The virus encodes three T (tumor) antigens that interact with various cell factors and growth signaling pathways. PyV-MT antigen is the major transforming protein that binds and activates protein kinase pp60 (*c-Src*) and other members of the *c-Src* family. PyV-MT antigen can transform cells by itself and is therefore a popular component of transgenic constructs for neoplasia research. Many transgenic lines of mice possess PyV-MT contributions to their genome.

Pathology

Under natural conditions, lesions are not likely to be encountered, except in immunodeficient mice. Nude mice have been shown to develop multifocal necrosis and inflammation, followed by tumor formation in multiple tissues reminiscent of experimentally inoculated neonatal mice. Microscopic examination of tissues from neonatally inoculated mice has revealed foci of virus replication in over 40 different cell types, underscoring the virus polytropism. Intranuclear inclusions can be observed with difficulty in cytolytic lesions and are most apparent in renal tubular epithelium (Fig. 1.20). Many of these foci give rise to transformed cells without virus replication. Cytopathic and proliferative changes are especially apparent in bronchiolar, renal pelvic, and ureteral epithelium. When genetically susceptible neonatal mice are experimentally infected with oncogenic strains of the virus, tumors arise most commonly from mammary gland, salivary gland, and thymus. Multiple skin tumors of hair follicle origin (with notable similarity to the skin tumors that occur in hamsters under natural conditions when exposed to hamster polyomavirus) are frequently observed. Tumors of mesenchymal origin are also common, including renal sarcomas, osteosarcomas, hemangiomas, and fibrosarcomas. Experimentally infected mice develop runting syndrome, polyarteritis, and enhanced autoimmune disease.

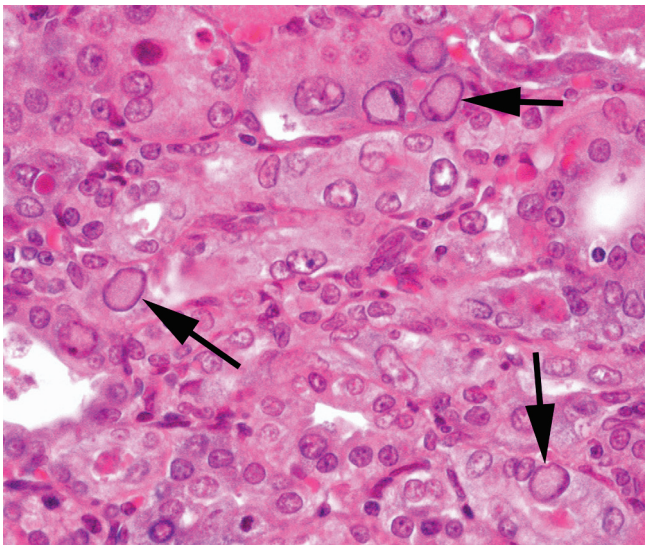

FIG. 1.20. *Intranuclear inclusions in the renal tubular epithelium (arrows) of a mouse experimentally infected with polyoma virus.*

Accidental infection of nude mice has been shown to result in multisystemic wasting disease, with paralysis and development of multiple tumors, particularly of uterus and bone. Experimental infections of nude mice yielded a very high prevalence of mammary adenocarcinomas among females and osteosarcomas among males. Nude mice can also develop infection of oligodendroglia with demyelination, similar to progressive multifocal leukoencephalopathy (PML) in immmunocompromised humans infected with BK and JC viruses, and macaques infected with SV-40 virus. Paralysis in nude mice is due to vertebral tumors as well as demyelination. A single report examined the effects of intraperitoneal inoculation of C.B-17-*scid* and B6-*scid* mice. Mice of both types became acutely ill and died within 2 weeks, with cutaneous hemorrhages and splenomegaly. The mice were thrombocytopenic, with depletion of

megakaryocytes in spleen and bone marrow, and developed marked extramedullary myelopoiesis in the spleen, which was misinterpreted as "myeloproliferative disease." If PyV gains access to other types of immunodeficient mice, it is likely to behave in a manner that is dictated by the properties of the virus, genetic background of the mouse strain, and nature of the immunodeficiency, but the rarity and inefficient transmission of PyV have limited the chances of natural exposure.

Diagnosis

The presence of PyV in immunocompetent mouse populations is best detected serologically, but PCR has been developed for detecting virus in tissues and other biological products. Differential diagnoses of nude mice with wasting disease include primarily mouse hepatitis virus, *Pneumocystis murina*, Sendai virus, and pneumonia virus of mice (PVM). Microscopic lesions containing intranuclear inclusion bodies must be differentiated from lesions caused by K virus, MAdV, and MCMV.

K Virus Infection

For all practical purposes, K virus is of historical interest and occurs rarely, if at all, in contemporary laboratory mouse colonies. Unlike PyV, K virus has no oncogenic action, either naturally or experimentally, in keeping with the absence of middle T antigen.

Epizootiology and Pathogenesis

K virus was initially discovered by Lawrence Kilham (thus the K) following intracerebral inoculation of infant mice with tissue extracts from an adult mouse during experiments on the mammary tumor virus. K virus is spread by the oronasal route. When orally inoculated into neonatal mice, the virus initially replicates in intestinal capillary endothelium and then disseminates hematogenously to other organs, including lung, liver, spleen, kidney, and brain, where it replicates in vascular endothelium. There appears to be strong tropism for pulmonary endothelium. At 6–15 days after inoculation of neonatal mice, there is a sudden onset of dyspnea due to pulmonary vascular edema and hemorrhage, resulting in rapid death. Pulmonary disease does not occur when older mice are inoculated, with complete resistance evolving between 12 and 18 days of age. Older mice mount an early and effective immune response that prevents the viremic phase of infection. Infection of nude mice results in disease similar to that seen in suckling mice. Regardless of age, mice remain persistently infected, and the site for virus persistence is renal tubular epithelium, which is typical of other members of the polyomavirus group. In naturally infected colonies, clinical signs are absent, with dams conferring passive immunity to litters during the disease-susceptible neonatal period.

Pathology

Gross lesions are restricted to lungs of neonatal or immunodeficient mice. Microscopically, intranuclear inclusions are present in vascular endothelium of jejunum, ileum, lung, liver, and occasionally brain. Inclusions are poorly discernible and require optimal fixation, especially in the intestine. Pulmonary lesions consist of congestion, edema, hemorrhage, atelectasis, and septal thickening. Livers of neonatal mice can have sinusoidal leukocytic infiltration and nuclear ballooning of cells lining sinusoids. Lymphocytic infiltrates (including interstitial pneumonia) arise in recovering mice. Inclusions may be found in renal tubular epithelial cells, frequently in groups of 2 or more, associated with foci of interstitial inflammation.

Diagnosis

Recognition of diagnostic lesions is difficult at best and is most likely in neonatally infected mice. Serological surveillance can be carried out by a variety of methods. PCR has been utilized to detect virus in infected mouse tissues. Differential diagnosis of multisystemic infection with intranuclear inclusions should include PyV, MAdV-1, and MCMV.

Poxvirus Infection: Ectromelia Virus Infection; Mousepox

No virus of laboratory mice conjures up an image of ruin like ectromelia virus (ECTV). Some of this reputation is justified, but most is human in origin. ECTV is a large DNA virus of the family Poxviridae and genus *Orthopox*, to which vaccinia, variola, monkeypox, cowpox, and others also belong. Orthopoxviruses share extensive antigenic cross-reactivity. Each is a distinct species, but the host range can be broad. Marchal reported an epizootic disease with high mortality in adult mice and termed it "infectious ectromelia," because of the frequency of limb amputation (ectromelia) in surviving mice. Frank Fenner performed seminal work on pathogenesis of the agent "ectromelia virus," which causes the disease "mousepox," although the terms are often erroneously interchanged. Outbreaks in the United States have stimulated renewed interest in the pathogenesis of mousepox. Mousepox was originally studied as a model for human smallpox, and that interest re-emerged with bioterrorism research.

Epizootiology and Pathogenesis

The origin of ectromelia virus remains an enigma, since it has never been found in wild populations of *M. musculus*. Unsubstantiated evidence has suggested infection of wild non-*Mus* rodents in Europe, but it has not been confirmed by appropriate sequence analysis and may actually reflect infection with cowpox virus. Ectromelia virus was at one time common among mouse colonies in Europe and may be enzootic in laboratory mice in China. In the past, outbreaks in the United States have been due to introduction of infected mice or mouse

products from Europe. In more recent outbreaks of mousepox, the source of the virus was traced to commercial mouse sera either collected from mice in the United States or imported from China. Strains of ECTV, including Hampstead, Moscow, NIH-79, Washington University, St. Louis 69, Beijing 70, Ishibashi I–III, and NAV, vary in virulence, but are serologically and genetically homogeneous. The NAV strain, which was isolated from mice infected with commercial mouse serum from China, is essentially the same virus as the original Hampstead virus. These findings strongly suggest that ECTV has not had a long or widespread enzootic history among commensal or domestic mice. ECTV is not highly contagious. It can be experimentally transmitted via a number of routes, but the primary means of natural transmission is believed to be through cutaneous trauma, which requires direct contact, and transmission has been shown to be facilitated by handling. Young mice suckling immune dams are protected by maternal antibody from disease but not from infection. ECTV readily infects the placenta and fetuses, but infected fetuses die and are not a source for vertical transmission within a population.

The hypothetical model of infection involves invasion through skin or mucous membranes, local replication, spread to regional lymph nodes, primary viremia, and then replication in spleen and liver. Between 3 and 4 days after exposure, a secondary viremia ensues, inducing replication of virus in skin, kidney, lung, intestine, and other organs. There is increasing evolution of lesions (disease) on days 7–11, including cutaneous rash. This scenario differs markedly between mouse genotypes. Susceptible mouse strains, such as C3H, A, DBA, SWR, CBA, and BALB/c, die acutely with minimal opportunity for virus excretion. Several other mouse strains develop illness but survive long enough to develop cutaneous lesions with maximal opportunity for virus shedding. Others, such as B6 and AKR mice, are remarkably resistant to disease and, therefore, allow inefficient virus replication and excretion. Thus, a textbook mousepox epizootic requires a select combination of introduction, suitable mouse strains for transmission, and the presence of susceptible strains for disease expression. Immunosuppression will exacerbate disease in mildly or subclinically infected mice. For these reasons, classic outbreaks of high mortality are often not seen in genetically homogeneous colonies of mice. Immunologically competent mice recover completely from infection and do not generally serve as carriers. Therefore, rederivation of virus-free mouse populations can be achieved from immmunocompetent mice. Immunodeficient mice cannot clear virus and are likely to be highly susceptible to fatal disease. Susceptibility to ECTV is dependent upon age, sex, strain, and immune status of the host and the virus strain. Interferons, NK cells, T cells, and B cells are all important. The genetics of resistance are complex and polygenic and not linked to *H-2* haplotype. Resistance

FIG. 1.21. *Healed amputating lesions of the distal extremities (ectromelia) from a mouse that survived natural infection with ectromelia virus. (Source: R. Feinstein, The National Veterinary Institute, Sweden. Reproduced with permission from R. Feinstein.)*

factors have been mapped to loci on chromosome 6 that includes the NK cell complex, chromosome 2 that includes the gene for C5, chromosome 17, and chromosome 1.

Pathology

Expression of lesions is dependent upon factors discussed above. Clinical signs range from subclinical infection to sudden death. External lesions during the acute phase of infection in susceptible surviving mice include conjunctivitis, alopecia, cutaneous erythema and erosions (rash), and swelling and dry gangrene of extremities, which result in "ectromelia" in surviving mice (Fig. 1.21). Internally, livers may be swollen, friable, and mottled with multiple pinpoint white to coalescing hemorrhagic foci (Fig. 1.22). Spleens, lymph nodes, and Peyer's patches may be enlarged, with patchy pale or

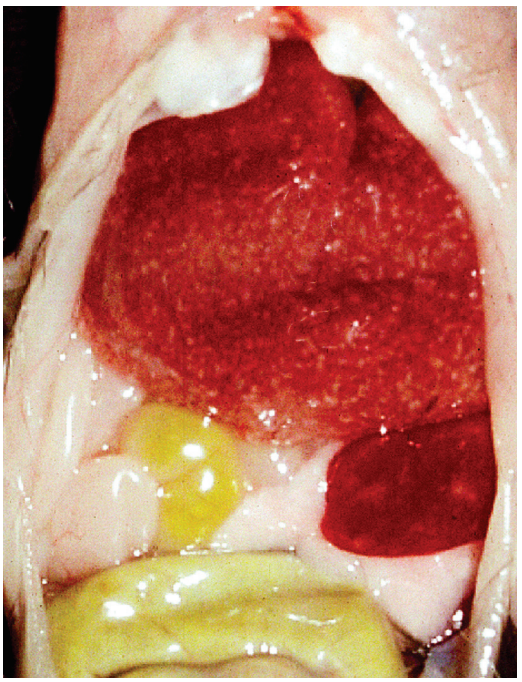

FIG. 1.22. *Multifocal necrotizing hepatitis and splenitis in a mouse during the acute phase of mousepox. (Source: Labelle et al. 2009. Reproduced with permission from American Association for Laboratory Animal Science.)*

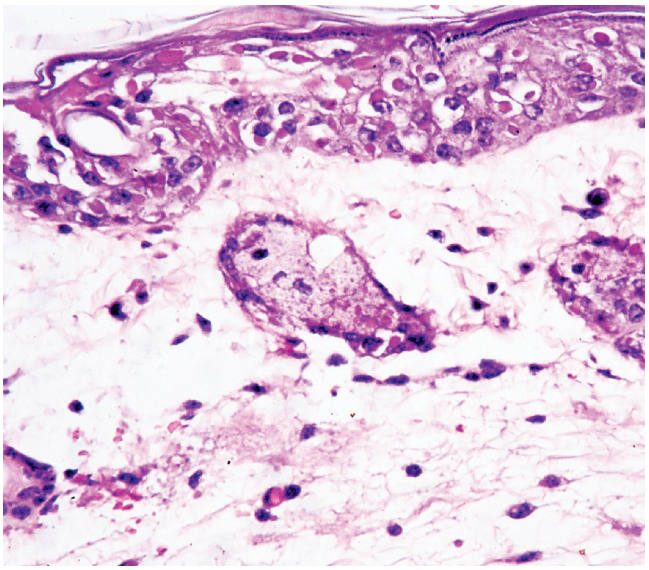

FIG. 1.23. *Skin from a mouse infected with ectromelia virus. Note ballooning degeneration and intracytoplasmic inclusions in the epidermis, with underlying dermal edema.*

hemorrhagic areas. Intestinal hemorrhage, particularly in the upper small intestine, is common. Microscopic lesions consist of focal coagulative necrosis in the liver, spleen, lymph nodes, Peyer's patches, and thymus, as well as other organs. Multiple basophilic to eosinophilic intracytoplasmic inclusion bodies (1.5–6 μm) are evident in infected cells, especially hepatocytes at the periphery of necrotic foci. These inclusions are poorly discernible with routine staining, but can be enhanced by doubling hematoxylin-staining time. Lymphoid tissue can be hyperplastic and/or focally necrotic, with occasional eosinophilic cytoplasmic inclusion bodies (type A pox inclusions or Marchal bodies). Erosive enteritis, often in association with Peyer's patches, is common, with type A inclusions in enterocytes. Skin lesions consist of focal epidermal hyperplasia, with hypertrophy and ballooning of epithelial cells and formation of numerous prominent large type A inclusions (Fig. 1.23). Later, skin lesions become erosive and inflammatory in character. Inclusions, inflammation, and erosion are also found in conjunctiva, vagina, and nasal mucosa. The conjunctival mucosa is a preferred area to search for inclusion bodies. Recovered mice often have fibrosis of the spleen and can have amputated tails and digits.

Diagnosis

The variable clinical signs and lesions can be problematic, but careful selection of clinically ill mice will enhance an accurate diagnosis. The complex of liver, spleen, and epithelial lesions bearing typical inclusions is pathognomonic. Splenic fibrosis in recovered mice is also a unique feature of this disease. Confirmation can be achieved by electron microscopic identification of the strikingly large poxvirus particles, immunohistochemistry, PCR, or virus isolation. Serology is a useful diagnostic adjunct in recovered mice and is an important surveillance tool for monitoring mouse populations. Serologic testing is likely to be of little value during the early stages of the infection. Vaccination is variably practiced and can interfere with interpretation of serology results. It should also be noted that vaccination protects mice from severe disease, but still allows active infection. Differential diagnoses must include agents that cause hepatitis in adult mice, such as MHV, Tyzzer's disease, salmonellosis, and others. Skin lesions must be differentiated from bite wounds, trichotillomania, hypersensitivity, and other forms of dermatitis. Gangrene and amputation of digits or tail can also occur due to trauma or "ringtail." Draconian depopulation measures to eliminate ECTV from mouse populations are probably not necessary if a rational approach to quarantine, testing, and rederivation is taken based upon the biology of this virus.

RNA VIRAL INFECTIONS

Arenavirus Infection: Lymphocytic Choriomeningitis Virus Infection

Mus musculus is the natural reservoir host for lymphocytic choriomeningitis virus (LCMV), and mice have carried this virus throughout the world from their original Old World niche. LCMV is a significant pathogen of humans, and was initially discovered during investigation of St. Louis encephalitis in 1933, when human brain material was injected into the brains of monkeys and mice, which developed lymphocytic choriomeningitis. The lesion, lymphocytic choriomeningitis, is not a feature of natural infection in mice. LCMV belongs to the family Arenaviridae, which includes a single genus *Arenavirus*, named because of the granular-appearing (Latin arenosus, "sandy") ribosomes within virions. If for no other reason, LCMV is important because of its significant zoonotic potential. LCMV has been studied extensively as a model system of immune-mediated disease, virus persistence, and immune tolerance, resulting in emphasis on aspects of infection that are not necessarily relevant to natural infection. LCMV has also been used as a model of noncytolytic viral disruption of differentiated cell function, resulting in disease without lesions (a claim that has not involved pathologists). LCMV is an unacceptable agent in laboratory animal facilities, and its eradication should be aggressively effected. The polytropic nature of LCMV and its wide host range allow this virus to readily infect transplantable tumors and cell lines, which can serve as a source of contamination for mouse colonies.

Epizootiology and Pathogenesis

LCMV is not ubiquitous, in that isolated mouse populations may or may not be enzootically infected. It is fortunately rare among contemporary laboratory mouse

populations, but may be a contaminant of biological products derived from mice. LCMV has also been found in pet mice and colonies of mice raised for feeding other species, including non-human primates. LCMV can naturally infect a variety of other mammals, including hamsters, guinea pigs, cotton rats, chinchillas, canids, and primates, including humans. Newborn rats can be infected experimentally, but this species seems to be refractory to natural infection. Among mice, the highly labile virus can be transmitted by direct contact and aerosol through nasal secretions as well as urine and saliva.

Enzootic infection in a mouse population is maintained by vertical transmission from dam to fetus and neonates. Infection of the fetus occurs during early pregnancy, and ova can be infected prior to implantation. There is no evidence for transmission by coitus. Nearly every cell in the fetus may become infected noncytolytically, with no significant adverse effect, although reduced litter sizes and runted pups may occur. The widely disseminated fetal infection involves the immature thymus, resulting in selective immune tolerance, with negative selection and depletion of LCMV-responsive CD8 T cells. This state of tolerance is highly LCMV selective, with otherwise normal immune responsiveness to other antigens. A similar scenario can occur if pups are infected as neonates. The state of LCMV immune tolerance allows multisystemic, persistent, subclinical infection, with the mice growing to reproductive maturity and perpetuating the cycle to the next generation. Immune-tolerant adult females that were infected as neonates can not only infect their own young in utero but also do not confer passive immunity to neonates, which further facilitates spread of the virus due to communal nursing behavior. Tolerance is not absolute, as mice develop LCMV-specific antibody, but antibody is non-neutralizing and complexed with excess viral antigen, which tends to be deposited in tissues, including arterial walls, choroid plexus, and glomeruli. Eventually, tolerance breaks down further, resulting in chronic lymphocytic infiltrates in multiple tissues and immune complex glomerulonephritis (late disease). The onset of late disease varies with genetic strain of mice. This phenomenon is irrelevant in wild mice, since mice become ill at an age in which their reproductive contributions to the population are no longer essential. LCMV immune-tolerant mice with disseminated infection can also develop a number of endocrine disorders due to diminished secretion of growth hormone with hypoglycemia, diabetes with hyperglycemia and abnormal glucose tolerance, and decreased thyroxine and thyroglobulin. These phenomenas are due to noncytolytic infection of endocrine organs, resulting in disturbance of cell function.

In contrast, natural or experimental infection of adult, immunocompetent mice follows a distinctly different course. Experimental infection results in a wide variety of disease manifestations, depending upon host and virus factors. Following natural exposure, or natural routes of experimental inoculation (intranasal or oral), immunocompetent adult mice typically develop short-term, acute infections from which they recover and seroconvert. However, parenteral inoculation with "aggressive" strains of virus results in disseminated infection of multiple organs, followed by a host immune response with CD8 T-cell-mediated disease. When virus is inoculated intracerebrally, disease is characterized by immune-mediated lymphocytic choriomeningitis (especially with "neurotropic" strains), whereas intraperitoneal inoculation results in immune-mediated hepatitis. In contrast, inoculation of mice with high doses of virus that are "docile" and "viscerotropic" results in immune exhaustion (in contrast to immune tolerance) and therefore mice develop no clinical, T-cell-mediated disease (thus, the term "docile"). The mechanism for immune exhaustion is selective targeting and high affinity for alpha-dystroglycan receptors on dendritic cells. The virus thus initially targets dendritic cells in the marginal zones of the spleen and lymph nodes, and then spreads to T-cell regions, with subsequent T-cell-mediated immune destruction of infected lymphoid tissue. This cycle of infection and destruction results in massive depletion of lymphoid tissues, including thymus, spleen, and T-cell regions of lymph nodes. This immune exhaustion results in global immunodeficiency, in contrast to the selective immune tolerance that takes place in fetally or neonatally infected mice. In both scenarios, virus infection is persistent.

LCMV strains and isolates cannot be differentiated serologically, as LCMV is a monotypic quasispecies. Nevertheless, there are a number of clonal laboratory strains with differing experimental tissue tropism and biological behavior, including Armstrong, Traub, WE, Pasteur, and others. Experimental disease is dependent upon virus strain, dose, route of inoculation, and host factors, including age, strain, and immunocompetence. A significant determinant of susceptibility to the adult, immune-mediated form of experimental disease is linked to the *H-2* locus. Mice with *H-2q/q* (e.g., SWR) or *H-2q/k* (e.g., C3H.Q) haplotypes are disease susceptible, whereas mice of the *H-2k/k* haplotype (e.g., C3H/He) are disease resistant. *H-2* haplotype is associated with CD8 T-cell responsiveness, but CD4 T cells, B cells, NK cells, and interferons, among other factors, contribute to LCMV immunity. The adult form of infection following parenteral inoculation is largely an experimental phenomenon, but it provides insight into the outcome of inoculation of mice with contaminated biological material. LCMV is a frequent contaminant of transplantable tumors. Considering the wide variety of immune-deficient mice being utilized today, and the many immune factors that are determinants of controlling outcome of infection or disease, awareness of the full spectrum of LCMV biology is useful.