Practical pathology of aging mice

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Old mice will have a subset of lesions as part of the progressive decline in organ function that defines aging. External and palpable lesions will be noted by the research, husbandry, or veterinary staff during testing, cage changing, or physical exams. While these readily observable lesions may cause alarm, not all cause undue distress or are life-threatening. In aging research, mice are maintained until near end of life that, depending on strain and genetic manipulation, can be upwards of 33 months. Aging research has unique welfare issues related to age-related decline, debilitation, fragility, and associated pain of chronic diseases. An effective aging research program includes the collaboration and education of the research, husbandry, and veterinary staff, and of the members of the institution animal care and use committee. This collaborative effort is critical to humanely maintaining older mice and preventing excessive censorship due to non-lethal diseases. Part of the educational process is becoming familiar with how old mice appear clinically, at necropsy and histopathologically. This baseline knowledge is important in making the determination of humane end points, defining health span, contributing causes of death and effects of interventions. The goal of this paper is to introduce investigators to age-associated diseases and lesion patterns in mice from clinical presentation to pathologic assessment. To do so, we present and illustrate the common clinical appearances, necropsy and histopathological lesions seen in subsets of the aging colonies maintained at the University of Washington.

Keywords: pathology; mice; aging; cancer; animal models; veterinary pathology

Aging is characterized by progressive declines in multiple organ systems with increases in many neoplastic and chronic degenerative non-neoplastic diseases. Mice are a popular mammalian species for aging studies as they are relatively economical to maintain for long-term aging studies and are similar to humans genetically and physiologically (1, 2). Genetically modified mice (GEMs) have been used extensively to test various hypothesis of aging (3, 4).

While maximum lifespan has been the mainstay metric of aging research, it is recognized that longevity alone, while informative, is incomplete as it does not allow for assessment of health. Physiologic, behavior, and pathology assessment coupled with the foundation of lifespan extension offer a more accurate characterization of a gene or intervention’s effect on maintenance of functional health. Pathobiology aging studies are now shifting toward characterizing health span and age-related disease onset in mouse models (5–7).

Defining what constitutes health and therefore health span in mice or humans is not a simple task. Medical experts with years of specialized training in geriatrics and/or biogerontology cannot completely define human healthy aging. For elderly mice, there is no predefined professional curriculum for specialized training, which is comparable to the human track. The mouse ‘geriatricians’ and biogerontologists are often a team of research scientists, veterinarians, medical doctors, graduate students, veterinary technicinan, and animal husbandry staff with varied educational and cultural backgrounds. To find a working consensus of a ‘healthy’ old mouse is challenging given the diverse backgrounds of the humans working with the mice. It is not a trivial consideration, either. Decisions on treatments and euthanasia for humane reasons are impacted by people’s expectations of what is a healthy mouse. Applying a definition proposed in M. Tatar’s report on the Biology of Aging Summit (6): ‘Good health is recognized by the ability to retain or return to normal levels when a subject is manipulated in a way to perturb a targeted system’, is one way to define health in a measureable way. However, ‘normal levels’ must be predefined, ideally, at the level of the cell, tissue, organ, organism, and colony. Individuals or groups working with the aging mouse colonies must have baseline information from which they can determine what is, and is not, ‘normal’ for the age of the mouse. If an observer has never seen a 30-month-old mouse, and is only familiar with the appearance of younger mice, they may be apt to classify an old mouse as ‘sick’ when it actually may only be exhibiting the expected...
age-associated frailty and readily observable but non-lethal lesions.

In this paper, we will provide practical overview of old mouse clinical (cage-level) presentations, necropsy, and histological findings in the UW aging mouse colonies. It is designed as an illustrative technical review to provide an introduction to the common disease patterns in older mice. The gross and histologic images are selected to exemplify the common lesions of old mice. It is not intended to be a comprehensive review of strain or gender-specific lesion spectrums. Rather, the aim here is to provide a foundation upon which readers can then build specific knowledge on the nuances of mouse models of aging. Disease spectrums in any one background strain, institution, gender, or genetically modified line (even those with identical background strains and diets) can and do vary (8). The importance of contemporary sex and aged matched wild-type littermate controls derived from the experimental colonies cannot be overstated (9).

Materials and methods

While this is a technical report, detailed methods covering animal housing, pathogen status, and clinical assessments of sick and moribund mice are pertinent as these variables impact phenotypes and vary between facilities. Reporting of such details is in compliance with the ARRIVE guidelines (10). Technical necropsy and histopathology methods are briefly covered for completion; the reader is referred to comprehensive reviews for additional information (11–13).

Animals and husbandry

The mice described herein were obtained from the C57BL/6J (Jackson Laboratories, Bar Harbor, ME) and CB6F1 aged mouse colonies housed at the University of Washington. Additionally, select gross photographs and histopathology examples were obtained from an unrelated inflammatory bowel disease research colony archives (Maggio-Price, University of Washington). In the aging colonies, animals were multiply housed (4–5 per cage, separate sexes) in ventilated cages containing Bed-O-Cob (Andersons, Maumee, OH) in a specific pathogen-free facility at the University of Washington. Mice were fed irradiated Picolab Rodent Diet 20 #5053 (PMI Nutrition International, Brentwood, MO) and provided reverse osmosis water. All supplies entering animal rooms were autoclaved, and rooms were maintained at 70/21°C, 45–55% humidity, with 28 air changes per hour and a 12-hour light/dark cycle. Sentinel mice were tested quarterly and were negative for endo-, and ectoparasites, mouse hepatitis virus, mouse parvovirus, and rotavirus, and they were tested annually for Mycoplasma pulmonis, pneumonia virus of mice, reovirus-3, Sendai virus, and Theiler’s murine encephalomyelitis virus. The mice from aging colonies mice were not tested for Helicobacter species or mouse Norovirus. The housing and experimental protocol for the two Helicobacter-infected mice (Fig. 1C–F) is described elsewhere (14). Historical (1980s) dermatitis and alopecia teaching archival cases presented in Fig. 2C,D lack complete strain and housing history. All animal procedures were approved by the University of Washington Animal Care and Use Committee.

Clinical assessments and definitions of sick versus moribund mice

Clinical observations in mouse colonies typically begin with superficial assessments by the husbandry staff during change changing. These readily conducted assessments include body condition, pelage condition, and activity levels (15, 16). A general consensus for normal mouse appearance includes a well-kept coat, bright eyes, erect ears, active and engaged in its environment (17). If an animal deviates from the observer’s definition of normal, it is reported to Veterinary Service Staff through generation of a ‘sick animal report’. Veterinary service staff and/or clinicians conduct a thorough physical exam and determine the health status of the mouse. Generally accepted sick rodent signs include hunched posture, unkempt coat, discharge from the eyes or other body orifice, rectal or uterine prolapses, skin lesions, palpable masses, reluctance to move, poor body condition, and/or hydration. The presentation of illness and its severity in a mouse can be varied and therefore descriptive criteria for end points must be established in order to minimize pain and distress and maximize data quality (15). For aging colonies, the mice are ideally maintained until near end of life (EOL) and will have age-associated diseases that may cause some to consider the old mouse ‘sick’(18). To prevent unwarranted censoring of mice due to non-terminal diseases, consensus descriptive criteria for end-of-life and humane euthanasia were developed. Mice were considered to be at EOL and euthanized when they were moribund and demonstrated one or more clinical signs suggestive of imminent death within 24 hours (19). These signs included (1) non-responsiveness to being touched, (2) cold body temperature to the touch, (3) slow or labored respiration, (4) hunched body position with matted fur, (5) failure to eat and drink (as determined by food hopper weights and degree of dehydration), or (6) poor body condition score or >20% loss of body weight relative to baseline (20). Mice that were nearing EOL, as determined by less severe clinical signs and up to 20% weight loss, were closely examined twice a day, 7 days a week. Mice that met the above criteria were killed by CO2 and necropsies performed.
Necropsy and tissue preparation

At necropsy, all major organ systems and any grossly abnormal tissues were preserved in 10% neutral phosphate-buffered formalin. Fixed tissues were trimmed using a standard protocol developed by the University of Washington Veterinary Diagnostic Laboratory. Bone tissues were decalcified and all tissues were routinely processed and paraffin embedded. Samples were sectioned at 4–5 μm and stained with hematoxylin and eosin.

For the cases described here, up to 30 tissues were examined using a protocol modified after Brayton et al. (12). Histological sections were examined and morphological diagnoses applied by PMT. Lesion definition and severity scoring systems are published elsewhere (21). Neoplastic diagnoses and terminology were based on consensus criteria (22). Special stains were employed in selected cases and included Movat’s pentachrome, Masson’s trichrome, or periodic acid Schiff’s. In some

Fig. 1. Gross and microscopic examples of rectal prolapses. A. Normal female 28-month-old B6 mouse with the anus indicated (arrow). B. Female 28-month-old B6 mouse with a mild acute rectal prolapse. Note the mild protrusion of congested and moist rectal mucosa (arrow). In female mice, rectal prolapse must be differentiated from uterine prolapse. C. Moderate to severe subacute rectal prolapse in a 4 to 6-month-old male genetically modified mouse, 129-Smad3tm/Par/J, infected with Helicobacter species. D. Chronic severe rectal prolapse in a 4 to 6-month-old male 129-Smad3tm/Par/J infected with Helicobacter species. The mucosa is dry and thickened with adherent crust. E. Hematoxylin and eosin-stained section of prolapsed rectum. The prolapsed mucosa is covered by a serocellular crust. Distal colon (DC) and rectoanal junction (box) are indicated. F. Higher power of boxed region in E. Squamous metaplasia (arrow) and hyperplasia of the rectal and distal colonic mucosa is common in chronically prolapsed tissues exposed to the cage environment including bedding, skin, and fecal organisms. The submucosa (SM) is markedly expanded by edema.
cases, complete blood counts with differentials and clinical chemistry were analyzed on blood obtained from the heart at necropsy (Phoenix Central Laboratories, Everett, WA).

**Review of pathology reports and databases**

Numbers of specific neoplastic and non-neoplastic diagnoses in aging B6 mice were determined by review of individual histopathology reports and databases. Non-neoplastic lesions were counted and tabulated by disease processes regardless of lesion severity. Prevalence was calculated as the number of mice affected. Table 1 has the numbers of mice with the histologic neoplastic diagnosis. Table 2 lists the non-neoplastic disease diagnosed either at cage level, necropsy, and/or histologically. Results were tabulated with sexes combined except where noted. A
comprehensive review of gender-specific lesion spectrums in a number of inbred strains, including UW aged C57BL6, will be published elsewhere. Complete blood counts with differentials and clinical chemistry panels for two of the mice are presented in Table 3.

### Results and discussion

Aging mice housed at the UW have four common clinical presentations, which are readily observable at the cage level and thus are frequently reported. These include rectal prolapse, alopecia and dermatitis, ocular lesions, and palpable masses. Whereas mice are reported and sometimes euthanized due to one of the above disease processes, there are often unsuspected comorbidities that may be more severe than the initial presenting clinical diagnosis. Many pathophysiological processes associated with aging, such as tissue atrophy, chronic inflammation, dysplasia, and occult neoplasia may not result in robust clinical signs (18). Additionally, moribund animals may be euthanized due to relatively non-specific signs such as hunched, cold to the touch with loss of body condition, and increased respiratory effort. The morbid state is often a result of the combination of diseases, especially in older mouse (21, 23). To characterize the full spectrum of lesions present at the EOL, and the definitive diagnosis of diseases, necropsies and histological examinations are required. Whereas clinical pathology including complete blood counts with differentials and chemistry panels are widely used antemortem diagnostic tests in most species, the limited amount of blood that can be humanely obtained from live mice restricts the number of tests that can be performed with any one sample. However, terminal blood collection usually provides an adequate

### Table 1. Survey of aged C57BL/6J colony neoplastic disease spectrum

| Neoplastic diseases                  | Location                        | % of mice with |
|--------------------------------------|---------------------------------|----------------|
| Fibrosarcoma                         | Uterus, skin, perirenal, ear    | 4              |
| Hemangioma                           | Spleen                          | 1              |
| Hemangiosarcoma                      | Spleen, uterus, ovary, liver, skin | 4            |
| Hematopoietic neoplasia (Malignant lymphoma or Histiocytic sarcoma) | Systemic                       | 67             |
| Hepatic adenoma                      | Liver                           | 1              |
| Leiomyosarcoma                       | Seminal vesicle, colon          | 3              |
| Malignant Pheochromocytoma           | Adrenal                         | 1              |
| Osteosarcoma                         | Vertebra                        | 1              |
| Ovarian granulosa cell tumor         | Ovary                           | 1              |
| Pituitary adenocarcinoma             | Pituitary                       | 1              |
| Pituitary adenoma                    | Pituitary                       | 6              |
| Pulmonary adenocarcinoma             | Lung                            | 3              |
| Pulmonary adenoma                    | Lung                            | 1              |
| Squamous cell carcinoma              | Skin                            | 1              |
| Squamous papilloma                   | Stomach                         | 3              |
| Testicular interstitial cell adenoma | Testicle                        | 1              |

**Note:** Retrospective review of clinical data, necropsy, and histology findings was tabulated. Results reported as percentage of mice with the disease ($n = 72$; males $= 26$, females $= 46$). Age range 16-36 months.

### Table 2. Survey of aged mouse colony non-neoplastic disease spectrum in C57BL/6J mice housed at the University of Washington

| Non-neoplastic diseases               | Location                        | % of mice with |
|---------------------------------------|---------------------------------|----------------|
| Acidophilic macrophage pneumonia      | Lungs                           | 13             |
| Amyloid, glomerular                   | Kidney                          | 41             |
| Amyloid, intestinal                   | Small intestine                 | 14             |
| Biliary hyperplasia                   | Liver                           | 3              |
| Cataracts or cornea opacity           | Eyes                            | 30             |
| Chronic infarct                       | Kidney                          | 4              |
| Cystic endometrial hyperplasia        | Uterus                          | 14             |
| Heart lesions$^a$                      | Heart                           | 97             |
| Hepatic cysts                         | Liver                           | 3              |
| Hydronephrosis$^b$                    | Kidney                          | 19             |
| Nephropathy                           | Kidney                          | 100            |
| Ovarian atrophy                       | Ovary                           | 6              |
| Ovarian cysts                         | Ovary                           | 3              |
| Polyarteritis                         | Systemic arteries               | 10             |
| Preputial cystic adenitis             | Preputial glands                | 3              |
| Rectal prolapse$^b$                   | Rectum                          | 19$^c$         |
| Seminal vesiculitis, cystic degenera- | Seminal vesicles                | 17             |
| Skin lesions$^d$                      | Skin                            | 17             |
| Systemic antigenic stimulation       | Most major organs               | 60             |
| Testicular degeneration               | Testes                          | 19             |
| Ulcerative keratitis                 | Eyes                            | 13             |

**Note:** Retrospective review of clinical data, necropsy, and histology findings was tabulated. Results reported as percentage of mice with the disease regardless of severity with a range of minimal to severe ($n = 72$; males $= 26$, females $= 46$). Age range 16-36 months.

$^a$Includes cardiomegaly, cardiomyopathy, arteriosclerosis, valvular lesions, and amyloidosis.

$^b$Diagnosed at cage level or at necropsy.

$^c$Out of total colony, $n = 711$.

$^d$Includes dermatitis and alopecia.
volume for full panels. Table 3 provides clinical pathology data and histopathological diagnoses for two of the mice illustrated in this manuscript. In the mice presented here, renal, vascular, inflammatory, and degenerative diseases were the common lesions diagnosed by histology. In the following section, the common age-associated diseases and lesions in the C57BL/6J colony at the UW are reviewed and illustrated.

**Rectal prolapse**

The mouse rectum, in contrast to the human, is extremely short and is susceptible to prolapse. Rectal prolapse (Fig. 1) in mice may be caused by inflammation in the colon due to infectious agents, such as pinworms or Helicobacter species (Fig. 1C/F), with increased abdominal pressure caused by pregnancy, masses, stranguria, or diarrhea. With age, it is plausible that the pelvic musculature may weaken allowing for prolapses to occur more readily – although this has not been investigated extensively (24). In many cases, the definitive cause for rectal prolapse is not determined; however, known causes may be excluded. In the UW aging colonies, pinworms and *Citrobacter rodentium* have not been diagnosed. The colonies are not tested for Helicobacter species, which can cause colitis and rectal prolapse in certain susceptible GEMs such as the 129-*Smad3tm/Par* model of bacterial-driven colon cancer (14). In immune competent mice, such as B6, Helicobacter species are considered commensal and should not cause disease. Rectal prolapses vary in clinical appearance (Fig. 1B-D) and, in female mice, must be distinguished from uterine or vaginal prolapse. Mild prolapse is a protrusion of the rectum extending only 1–2

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**Table 3.** Clinicopathological data from B6 mouse presented in Figures 2B, 5C, and 5D and CB6F1 mouse presented in 3B, 3D, and 4C

|                     | B6 Female Figs. 2B and 5C,D | CB6F1 Male Figs. 3B, D and 4C | Reference values a |
|---------------------|-----------------------------|-----------------------------|-------------------|
| **Complete blood count** |                             |                             |                   |
| WBC, K/ul           | 2.5                         | 14.8                        | 5.1–11.6          |
| RBC, M/ul           | 8.5                         | 11.1                        | 8.7–10.5          |
| HGB, g/dl           | 12.9                        | 15.4                        | 12.2–16.2         |
| HCT,%               | 51.0                        | 55.8                        | 42–44             |
| MCV, fl             | 60.1                        | 50.1                        | na                |
| MCH, pg             | 15.3                        | 13.8                        | na                |
| MCHC                | 0.3                         | 27.6                        | na                |
| Platelet count, K/ul| 992.0                       | 2043.0                      | 100–1,000         |
| **Differential**    |                             |                             |                   |
| Bands               | 0 (0.0)                     | 0 (0.0)                     | 0                 |
| Polys               | 960 (38.0)                  | 6,220 (42.0)                | 6.7–37.2          |
| Lymph               | 1,240 (49.0)                | 7,090 (48.0)                | 63–75             |
| Monos               | 250 (10.0)                  | 1,030 (7.0)                 | 0.7–2.6           |
| Eos                 | 80 (3.0)                    | 440 (3.0)                   | 0.9–3.8           |
| Baso                | 0 (0.0)                     | 0 (0.0)                     | 0–1.5             |
| **Comments**        | Polychromasia, +1           | Slight polychromasia        | Platelet count inaccurate due to clumps |
| **Small mammal panel** |                             |                             |                   |
| Glucose mg/dl       | 196.0                       | 170.0                       | 106–278           |
| Urea nitrogen (BUN), mg/dl | 24.0              | 30.0                        | 19–34             |
| Calcium, mg/dl      | 10.6                        | 10.1                        | 9–12              |
| Total protein, g/dl | 6.9                         | 5.7                         | 4.3–6.4           |
| Albumin, g/dl       | 3.5                         | 3.2                         | 2.0–4.7           |
| Alanine aminotransferase, U/l | 43.0 | 47.0                   | 26–120            |
| Aspartate aminotransferase, U/l | 83.0 | 107.0                 | 69–191            |
| **Principal histologic diagnoses** | Pituitary adenocarcinoma | Harderian gland adenoma |
|                     | Histiocytic sarcoma         | Preputial adenitis and seminal vesiculitis |
|                     | Keratitis and corneal ulceration | Mild nephropathy |
|                     | Polyarteritis               |                             |

aSummarized from (58, 59).
mm from the base of the tail and perineum and the prolapsed mucosa is light pink and moist, with little adherent material (Fig. 1B). Mild prolapse often cause little detriment to the animal, which can survive and thrive with these lesions for some time. Severe rectal prolapse is a protrusion of both distal colon (DC) and rectal mucosa extending >3 mm from the base of the tail and perineum (Fig. 1C,D). The severely prolapsed mucosa is often reddened and edematous to necrotic with adhered fecal or bedding material (Fig. 1C). Often, feces inside the cage will be sticky, an indication of diarrhea. In long-standing cases, the mucosa may become proliferative (Fig. 1D). Exposure of the delicate colorectal mucosa to the cage environment provides a nidus of chronic inflammation and entry of bacteria in the systemic circulation. There is no completely effective treatment for rectal prolapse. Clinical recommendations for mice with rectal prolapses vary between facilities and experimental protocols; in general, mild rectal prolapses with no other clinical signs of systemic illness are often monitored, while euthanasia is recommended for mice with moderate to severe rectal prolapses.

Histologically, the prolapsed mucosa may be ulcerated to necrotic or, with chronicity, proliferative with squamous metaplasia (Fig. 1E,F). Adherent bacterial colonies, plant material and fecal matter may also be present. In typical cases, the underlying vasculature and submucosa (SM) is edematous and inflamed and may be congested. Inflammation may be acute, primarily neutrophilic when there are ulcers and bacteria, or lympho-
histiocytic when chronic. Chronic prolapse can lead to severe mucosal hyperplasia with subsequent mucosal herniation due to the thin SM. The herniated glands must be differentiated from invasive carcinoma (25).

**Skin lesions**
Barbering, alopecia, dermatitis, and scarring are commonly seen in mice (Fig. 2). Dermatitis may have a variety of causes including infectious agents, such as fur mites, or fighting with secondary bacterial infections. These will often respond to treatments such as removal of aggressive mice, topical and oral antibiotic, or corticosteroids (26). In contrast, idiopathic ulcerative dermatitis in the B6 mouse does not respond to treatment. Affected B6 mice are usually pruritic leading to self-mutilation, disease progression and, often, euthanasia for humane

Fig. 4 (Continued)
reasons. The B6 chronic ulcerative dermatitis has been attributed to many causes (27, 28) most recently primary follicular dystrophy (29). The clinical disease is variable, waxes and wanes, appears to have sex, and season predilections. As seen with other strain-specific diseases, the use of F1 hybrids has a decreased incidence of B6 dermatitis (1, 30); however, skin lesions remain a significant background factor in aging colonies (29). Collectively, skin lesions in aging mouse colonies have variable presentations ranging from the very mild alopecia to severe ulcerative disease requiring euthanasia. Mild changes, such as ill-kept fur (Fig. 2B) are often one of the first clinical signs noted in diseased mice. It is nonspecific and a result of decreased grooming. Alopecia in mice may present on the head (Fig. 2A) or in a patches (Fig. 2C). Barbering by cage mates may result in areas of hair loss; however, distinctive patterns (Fig. 3A) and the presence of one unaffected (dominant) mouse in the cage will help to rule out dermatitis. Histologically, dermatitis is also variable in presentation and severity, depending on etiology and chronicity. Mild lesions (Fig. 2E) with few scattered inflammatory cells may be noted in regions of clinical alopecia. Severe lesions may occur and are often a combination of ulceration, necrosis, and associated chronic-active inflammation. Healing is by dermal fibrosis and marked epithelial hyperplasia (Fig. 2F). Like rectal prolapses, open skin lesions serve as a nidus for bacterial infection with skin commensals (Staphylococcal species) and result in smoldering inflammation that may affect physiological parameters such as inflammatory cytokines, leukograms, lymphadenopathy, and reactive amyloidosis (31).

Ocular lesions
The cornea of mice is relatively large compared to humans and is exposed to exogenous irritants such as bedding dust and ammonia levels, which can cause contact keratitis. Skin commensals may also contribute to keratitis, conjunctivitis, and blepharitis. With age, atrophy and chronic inflammation of the lacrimal glands is very common and may result in decreased tear production and secondary corneal lesions (keratoconjunctivitis sicca). Regardless of the cause, corneal lesions appear as opaque eyes with variable amount of discharge. In pigmented strains, corneal opacity is particularly easy to recognize due to the contrast with the dark globe (Fig. 3C). Ulcerative keratitis may be diagnosed clinically via ophthalmic exams and fluorescein stain uptake into the ulcerated cornea. Treatment options may include antibiotics and/or steroids and will vary by facility and experimental protocol. Frequently in aged mice, the bulk of the ulceration and active inflammation is resolved; histologically, the chronic corneal lesion is characterized by proliferation of the corneal epithelium with varying neovascularization and chronic inflammation (Fig. 3E,F). Other eye lesions commonly reported include proptosis with or without bulopthalmia secondary to retrobulbar abscesses, Harderian gland, or intraorbital gland neoplasias (Fig. 3B,D). Cataracts are also quite common in aged mice but are more challenging to diagnose clinically usually requiring slit lamp examination (32, 33) antemortem or postmortem histopathological assessment.

Palpable masses
Subcutaneous or internal palpable masses may be due to neoplasia or abscesses, possibly both, and are common in aged animals. Affected mice present with irregular body contours and may have decreased mobility if the masses are large or in the inguinal or axillary areas (Fig. 4A,B). In the UW aging colonies, enlarged and occasionally infected seminal vesicles in male mice were reported

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initially as abdominal tumors (Fig. 4A,B). In older B6 male mice, progressive enlargement of the seminal vesicles is extremely common (34) and our experience suggests that it is also common in CB6F1 hybrids.

Subcutaneous masses in the inguinal or pelvic region often were enlarged cystic or infected preputial or clitoral glands (Fig. 4E,F). The subcutaneous lymph nodes, especially the submandibular, may be enlarged due to
physiologic lymphoid reactive hyperplasia secondary to systemic or localized inflammation. Systemic lymphadenopathy involving subcutaneous and internal lymph nodes is most frequently due to malignant lymphoma. Other subcutaneous structures such as the mammary fat pads or salivary glands may also become inflamed or neoplastic and present as palpable masses. Often, the masses can be diagnosed as inflammatory or neoplastic at necropsy with the combination of the gross presentation and cytology. However, histopathology is definitive as neoplasia may also be infected or inflamed. For example, the retro-orbital mass in Fig. 4C was suspected clinically, and at necropsy, to be a chronic retrobulbar abscess. Final histopathological diagnosis was a Harderian gland adenoma (Fig. 3D). For malignancies within the lung, determining whether neoplastic masses are primary lung tumors versus metastatic disease may be challenging without the aid of histology (Fig. 4G,H). Inbred strains vary in their typical spectrum of disease and neoplasia is no exception (9, 35, 36). In the B6 background, hematopoietic neoplasias (malignant lymphoma and histiocytic sarcoma) are extremely common (Table 1) (21, 36, 37). Necropsy findings for these hematopoietic neoplasias often overlap, for example, enlarged mesenteric lymph nodes (Fig. 4I) can be affected by either type. While there are differences in organ distribution and cellular morphology (Fig. 5I–L) that allow distinction between the malignant lymphomas and histiocytic sarcoma, definitive diagnosis may require immunohistochemical or molecular analysis (38–40).

Lesions diagnosed at necropsy or histologically

Frequently, internal lesions not detected at physical exam, will be observable at necropsy. For example, in a mouse with labored respiration, the lungs may contain pulmonary tumors with an enlarged heart (Fig. 4G, H). A mouse reported with head tilt or circling may be submitted to necropsy to rule out central nervous system disease, but is more likely to have otitis media/externa or arteritis impacting the vestibular system (31). Neurological signs may be due to compression of brain tissue by intracranial neoplasias such as pituitary adenoma/adenocarcinoma (Fig. 5C,D). Splenomegaly is a frequent non-specific necropsy finding that can be due to extensive extramedullary hematopoiesis, which occurs as a physiological response to stimuli such as chronic inflammation or neoplastic diseases (31). With either cause, the spleen can be markedly enlarged (41). Histology can aid in definitive diagnosis of gross lesions that have multiple differential diagnoses.

Complete histopathological examination often reveals more disease processes than were suspected clinically or diagnosed at necropsy. These microscopic morphological data offer insight into unexpectedly interesting or confounding covariates. Necropsy and pathology also serves to confirm the absence of excluded agents, for example, internal and external parasites or evidence of mouse coronavirus (mouse hepatitis virus). Table 2 summaries the microscopic non-neoplastic diagnoses and histological examples are presented in Fig. 6 and Fig. 7. Common non-neoplastic diseases diagnosed histologically include renal disease, arteritis, systemic inflammation, and degenerative lesions.

Renal disease

Renal lesions are common in older mice and include chronic nephropathy, glomerular amyloidosis (42), glomerulonephropathies (43), and obstructive uropathy (44). Kidneys with chronic nephropathy and or glomerular amyloid (if severe) appear pale and pitted and the size may be altered. Cysts, infarcts, and hydronephrosis all may impact kidney size and shape. Obstructive uropathy is common in male mice and is due to blockage of the lower urinary tract from multiple causes (44). Retention of urine may result in or from inflammation of the urogenital tract or accessory sex glands and pressure may cause hydronephrosis (Fig. 4E). Histologically, chronic nephropathy is characterized by early tubular lesions and progresses to include glomerular changes and interstitial inflammation (Fig. 6C,D). There is a large reserve capacity of the kidney to maintain function even in the face of nephron loss thus, for nephropathy alone to cause significant morbidity, it would have to be moderate to severe or accompanied by other lesions affecting the
kidney such as hydronephrosis or neoplasia. However, subclinical or mild nephropathy with associated diminished renal reserve would increase susceptibility to additional insults and homeostatic imbalance (45). Glomerular amyloidosis and membranous glomerulonephropathy may appear histologically similar and can be differentiated by use of Congo Red for amyloid and PAS for increased mesangial matrix (31).

Fig. 6 (Continued)
**Arteritis**

Segmental arteritis with variable components including fibrinoid necrosis, smooth muscle proliferation, and mixed inflammation occurs in small to medium arteries of various organs (Fig. 6I,J) (31). Commonly affected tissues in these studies included the mesentery, tongue heart, reproductive tracts, and kidney. An immune mediate pathogenesis has been proposed (27, 46). In humans with a similar disease processes, polyarteritis nodosa, clinical signs are related to affected organ dysfunction (47). In mice, the most overt clinical sign associated with arteritis is vestibular syndrome (head tilts and/or circling) as describe above (31).

**Chronic inflammation**

As a secondary process of many diseases associated with aging in mice, and consistent with reports of a proinflammatory state in elderly humans (48, 49) and mice (19), histologic evidence of systemic immune stimulation is present in numerous tissues. Findings include reactive lymphoid hyperplasia in spleen and lymph nodes, increased lymphoid aggregates in various organs, expansion of mesenteric/omental milk spots, acidophilic macrophage pneumonia (AMP), reactive amyloidosis, and the renal lesions discussed above. Omental milk spots (50), mesenteric aggregates with mixed lymphocytes, histiocytes, and plasma cells are frequently reactive in older mice with germinal centers and occasional Mott cells (Fig. 6A,B). Similar reactive changes can be present in lymphoid aggregates found in various tissues including the renal pelvis, bladder, lungs, and liver. Reactive lymph nodes may present clinically as a lymphadenopathy with histologic sinusoidal histiocytosis, mastocytosis, or plasmacytosis with germinal centers, which aid in differentiation by hyperplasia from neoplasia. AMP is a common idiopathic disease in mice, especially of those on B6 and 129Sv background (51, 52). Although definitively diagnosed with histopathology, severe AMP may be noticed at necropsy as foci of firm, pale tan to cream areas in the lungs, which fail to collapse. The AMP is often associated with pulmonary neoplasia (Fig. 5A,B) or chronic pneumonia (Fig. 6K,L) (31, 53). The characteristic histopathologic lesion is accumulations of large macrophages with abundant cytoplasm containing variable numbers of bright eosinophilic crystals. Multinucleated giant cells, epithelial hyalinosis, and extracellular acicular crystals may also be present (51).

**Miscellaneous degenerative lesions**

Dysplastic and degenerative dental and joint lesions are presented in Fig. 7 as examples of subclinical histologic disease, which likely contributes significantly to the decline in functional health of the affected mouse and may be under diagnosed. We and others (54) have noted dysplastic intrapulpal denticles in the incisors of older mice (Fig. 7A,B). These lesions may result in abnormal wear and/or breakage of the incisors that contribute to periodontitis and decreased ability to effectively gnaw rodent chow. We have noted, in two of three cases of intrapulpal denticles, an association with moderate to severe temporomandibular osteoarthritis (Fig. 7C,D). The precise relationship between the development of these two lesions is unknown – whether the abnormal tooth development aggravated the joint disease or vice versa. It is plausible that the combination of the lesions results in painful and ineffective mastication. Osteoarthritis of the knee, hip, elbow, and vertebra would result in abnormal gaits and, if severe, clinically recognizable joint swelling due ossification of the joint capsule and periarticular fibrosis (55). Preliminary necropsy results from the current health span study in CB6F1 suggest the incidence of clinical osteoarthritis may be relatively high (personal observation CPB). Other preliminary observations from necropsies in the on-going health span study include high incidence of skin, male reproductive tract, renal lesions, and hepatosplenomegaly that likely is due to hematopoietic neoplasia.

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**Fig. 6.** Histological presentations of subclinical chronic systemic inflammation-associated lesions seen in C57BL/6 mice aged 16–36 months. A. Hyperplastic mesenteric milk spot. B. Higher magnification of A. The reactive milk spot contains large and small lymphocytes, plasma cells, and Mott cells (arrows). C. Low magnification overview of chronic renal lesions. Interstitial lymphoid aggregates (*) and tubular ectasia (arrow) with degeneration, regeneration, necrosis and membranous glomerulonephropathy. D. Thickened mesangial matrix (*) and periglomerular fibrosis (arrow) characterize membranous glomerulonephropathy. Glomerular amyloidosis should also be considered. PAS and Congo Red histochemical stains can aid in differentiation. E. Chronic adenitis occurs in numerous glands including the exorbital lacrimal gland. F. Higher magnification of E. Lymphoplasmacytic inflammation disrupts the gland architecture. Note ectatic duct (*). G. Amyloid accumulation in the small intestinal villi. H. Higher magnification of G. An accumulation of light pink homogenous extracellular material (amyloid) in the lamina propria widens the villi. I. Cross-section of the tongue with poly arteritis nodosa (box and *). Lingual minor salivary glands are indicated (arrow). J. Movat’s pentachrome staining of lingual artery in I. The affected segment (*) lacks in elastic lamina (thin black line) and the media is expanded by smooth muscle cells. Mild to moderate perivascular chronic inflammatory cells (arrow) and lumena (L) are indicated. Similar necroproliferative arteritis near the inner and middle ear may result in neurological signs (see text for details). K. Low power view of lung severely affected by AMP. L. Higher magnification of AMP demonstrates the intrahistiociytic crystalline material (*) and dense perivascular and peribronchioral lymphohplasmacytic inflammation, which is frequently present in severe cases. Hemosiderophage (arrow) and hyalinized respiratory epithelium (arrowheads) are indicated.

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In older humans and mice the incidence of chronic disease increases and with it the intensity of clinical care and monitoring. Intensive monitoring of aged mice is needed to prevent undue suffering and loss of valuable data when mice are found dead (18, 19). On the other hand, excessive censoring of mice due to easily observable external non-lethal lesions can impact aging studies (21). A balance is needed and can be achieved via education about the unique appearances and humane care issues associated with geriatric mice (19, 56, 57). Here we have illustrated some of the common clinical, necropsy, and histologic lesions in aged mice to provide a basic introduction into the variety and complexity of age-associated diseases in mice. Necropsy and histopathologic assessments of aged mice coupled with antemortem physiologic testing allows for full characterization of disease onset and patterns, thereby defining frailty and decline in function in aging mouse models. Validation of these models so that they may be better translated to human aging interventions is the goal for the entire biogerontology community of geriatricians, gerontologists, basic scientists, medical and veterinary scientists, and pathologists (5, 6).

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