Survival and Lung Pathology of Mouse Models of Hermansky- Pudlak Syndrome and Chediak-Higashi Syndrome (44359)

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Abstract. **Hermansky-Pudlak Syndrome (HPS), a recessively inherited disease in humans, affects the biosynthesis/processing of the related intracellular organelles: lysosomes, melanosomes, and platelet dense granules. The disease is multigenic in both humans and mice where 14 separate genes have been demonstrated to be causative. Patients often die prematurely with severe lung abnormalities. Patients with the related Chediak-Higashi Syndrome (CHS) likewise have significantly reduced life spans. Long-term survival and lung histomorphology were analyzed in a pilot experiment involving several genetically defined singly and doubly mutant mouse HPS mutants and the beige CHS mutant to determine whether these parameters are altered in the mouse models. The mutants differed widely in both longevity and lung architecture. Mice doubly homozygous for the pale ear and ruby eye or for the muted and pearl genes had the shortest life spans with none surviving the two-year experimental duration. Life spans were similarly severely reduced in the beige and gunmetal mutants. Intermediate life spans were apparent in the pearl, pallid, and cocoa mutants whereas minimal effects were noted in ruby eye, muted, light ear, and cocoa mutants. Enlarged air spaces were noted in histologic sections of lungs of several of the mutants. For the most part, the severity of lung abnormalities was inversely proportional to the long-term survival of these various mutants, suggesting that lung pathology may contribute to mortality, as has been suggested for human HPS patients.**

[P.S.E.B.M. 1999, Vol 220]

Human Hermansky-Pudlak Syndrome (HPS) is an inherited disease affecting the three related subcellular organelles: melanosomes, platelet dense granules and lysosomes (1–3). It was first described in 1959 by the Czechoslovakian doctors Hermansky and Pudlak (4), and affected individuals present with oculocutaneous pigment dilution, prolonged bleeding times because of the platelet granule functional defect and accumulation of ceroid aging pigment in lysosomes. Clinically, the more important feature of the disease is the eventual occurrence, in a significant percentage of HPS patients, of fibrotic restrictive lung disease (2). The lung disease produces significant morbidity in mid-life. Associated clinical problems include hemorrhaging, colitis, and in rare cases, kidney failure. No curative therapies exist.

Chediak-Higashi Syndrome (CHS) is closely related to HPS in that it is also a disease affecting the same three subcellular organelles (5). A major difference is that giant granules are found in most cells. Also, it is even more deadly than HPS with affected individuals typically dying in their teen years though recently bone marrow transplantation has proved therapeutic in some patients (6).

Mouse models for both HPS and CHS have been characterized. Fourteen genetically distinct mouse HPS-like models (3) are found among the large group of mouse hypopigmentation mutants. In addition to hypopigmentation, all have prolonged bleeding times due to platelet granule

This work was partially supported by shared resources of the Roswell Park Cancer Center Support Grant (P30 CA16056) and by NIH grants HL51480, HL31698 and EY12104.

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Received June 16, 1998. [P.S.E.B.M. 1999, Vol 220]
Accepted November 9, 1998.

0037-9727/99/2203-0162$10.50/0

abnormalities (platelet storage pool deficiency), and most have lysosomal secretion deficiencies and/or accumulation of ceroid in lysosomes. The pale ear mouse has been demonstrated to be the genetically appropriate mouse model for the form of HPS found in Puerto Rico (7, 8). Because of recent evidence of genetic heterogeneity of human HPS (9–11), it is apparent that other animal models will be needed to identify orthologous genes and to accurately model human HPS. The beige mouse is an appropriate model for human CHS, though other animal CHS models also exist (5, 12).

Decreased survival is apparent in both HPS and CHS patients. Accordingly, we have in this pilot experiment, analyzed the long-term survival of several of the mouse HPS models together with the beige CHS mouse. Also, future usefulness of the mouse models depends upon the presence of some of the critical clinical abnormalities, such as the lung pathology, seen in older HPS patients. A preliminary analysis of lung morphology has therefore been conducted by light microscopy in selected aged HPS mice.

Materials and Methods

Animals. All mutant and control mice were originally obtained from The Jackson Laboratory and were subsequently bred and maintained at Roswell Park Cancer Institute. Mice were housed in microisolator cages which were changed weekly. Most mutant stocks (pearl, ruby eye, beige, gunmetal, light ear and pallid) occurred as spontaneous mutations on, or were transferred to, the C57BL/6 J inbred strain, by repeated backcrossing (3). Muted mice were originally obtained from The Jackson Laboratory on the CHMU/Le *ch* +/+ *mu* background (13) whereas cocoa (14) was obtained on the C57BL/10 J background. Each hypopigmentation mutation is recessive. Therefore, the mutant stocks were homozygous for the mutant allele. The 22 control mice used in this study included 15 C57BL/6 J and 7 heterozygous +/*mu* mice on the CHMU/Le background.

Use of animals is with the review and approval of the Roswell Park Institutional Animal Care and Use Committee.

Long-Term Study. Four male and four female mice, homozygous for each pigmentation mutation, were chosen to initiate the study. Control mice were likewise equally divided between male and female mice. Animals were maintained in static microisolators (Allentown Caging, Allentown, NJ) and given Teklad 6% rat and mouse diet and acidified water *ad libitum*. Cages were changed once per week. Bedding was BED O' COBS (The Andersons Industrial Products Group). Mice were carefully visually inspected twice per week during the course of the 2-year period. Only those mice that could be physically accounted for as having died or that were sacrificed due to a moribund condition were included in the final survival curves. Mice were necropsied when sacrificed. Mice that were found dead were subjected to macroscopic external and internal examination.

Statistical comparisons of longevity were performed by a two-sided Cox-Mantel test using the BMDP1 L software package (SPSS Inc., 444 Michigan Ave., Chicago, IL).

Histology. At necropsy, mice were examined grossly for apparent abnormalities, tumors, and other pathology. Obvious lesions were dissected and, along with normal tissues, fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 μ and stained with hematoxylin and eosin for microscopic examination. Illustrated sections are representative of at least three separate mutant mice. Peripheral blood films were taken at 6–8-week intervals and examined (Leukostat, Fisher Diagnostics, Fisher Scientific, Pittsburgh, PA) for evidence of leukemia and other gross hematological abnormalities.

Results

The survival of the HPS and CHS mouse mutants was monitored over a 2-year period (Fig. 1–3). Survival comparisons in which *P*-values are significantly different ($P < 0.05$) are given in the legends. The greatest impacts on life spans were observed in those stocks doubly homozygous for two HPS genes; either muted and pearl (Fig. 1A) or pale ear and ruby eye (Fig. 1B). None of the seven muted-pearl nor five pale ear-ruby eye mutants survived the full 2 years. Loss of muted-pearl mice commenced at early times (4–6 months) whereas all pale ear-ruby eye mice survived until 16 months at which point there was a precipitous loss of viability. In contrast only 1 of 22 control mice died spontaneously during the 2-year study. This was a *mu*/+ mouse, and no obvious pathology or cause of death of this mouse was apparent upon macroscopic examination.

Although the combination of muted and pearl genes drastically lowered viability, lesser effects of these mutated genes were apparent when present singly (Fig. 1A). In fact, all five muted mice survived for 2 years whereas five of eight pearl mice survived this period. Seven of eight ruby eye mice survived the full 2 years. The viability of the other single mutant, pale ear, was not determined because mice with this mutation were not available in our colony at the initiation of this study.

Long-term survival of most singly mutant mice (with the exception of the muted mutant) was intermediate between that of control mice and double mutants. The greatest effects were in beige and gunmetal (Fig. 2); only two of seven and one of five mutants, respectively, survived the full two years. Intermediate long-term survival rates of 50%–80% were noted for pearl (Fig. 1A), cocoa (Fig. 3) and pallid (Fig. 3). These reductions in longevity were significantly less than that of controls. At the opposite extreme, lack of or minimal effects on longevity occurred in muted (Fig. 1A), light ear (Fig. 3), and ruby eye (Fig. 1B), whose long-term survivals were not statistically different from controls.

By microscopic examination, lung tissue of several HPS mouse mutants, sacrificed at the end of the 2-year study, appeared abnormal, though the severity of the effects differed (Fig. 4). Representative sections of several mutants

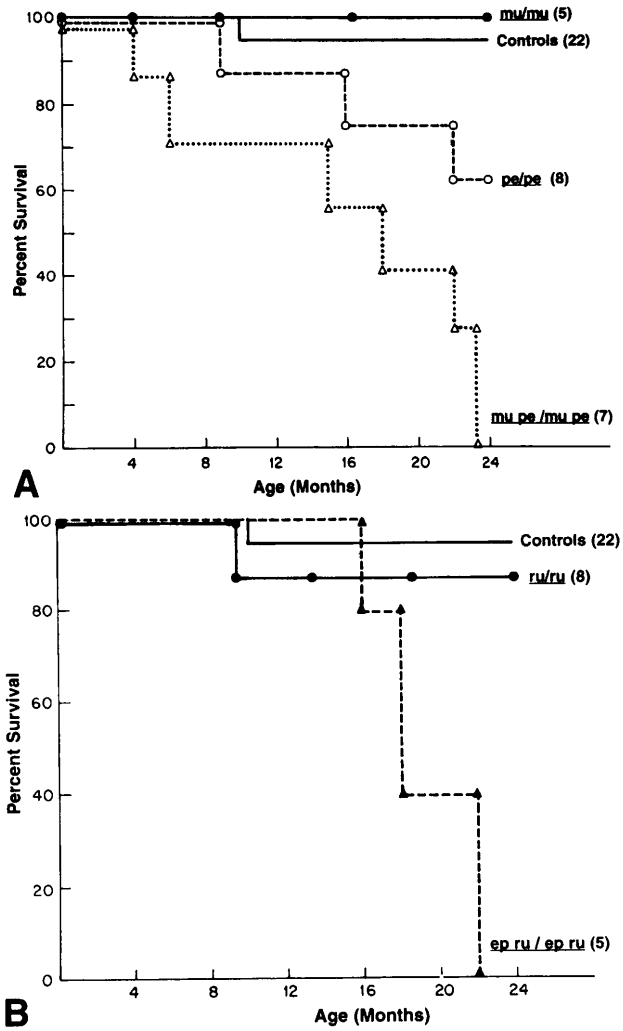

Figure 1. Long-term survival of mutants doubly homozygous for HPS genes. (A) Survival of mutants doubly and singly homozygous for the muted *(mu)* and pearl *(pe)* genes together with control mice. (B) Survival of mutants doubly homozygous for the pale ear *(ep)* and ruby eye *(ru)* genes together with those homozygous for the ruby eye gene. The number of mice in each group are indicated in parentheses. *P*-values: *epru/epru* vs. controls (< 0.0001); *epru/epru* vs. *ru/ru* (< 0.0442); *mupe/mupe* vs. controls (< 0.0001); *mupe/mupe* vs. *mu/mu* (< 0.0015); *mupe/mupe* vs. *pe/pe* (< 0.0223); *pe/pe* vs. controls (< 0.0188).

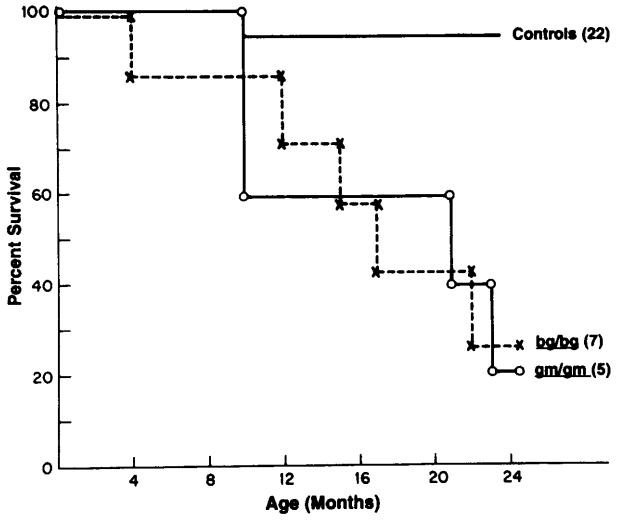

Figure 2. Long-term survival of mutants singly homozygous for the beige *(bg)* and gunmetal *(gm)* genes. *P*-values: *bg/bg* vs. controls (< 0.0001); *gm/gm* vs. controls (< 0.0001).

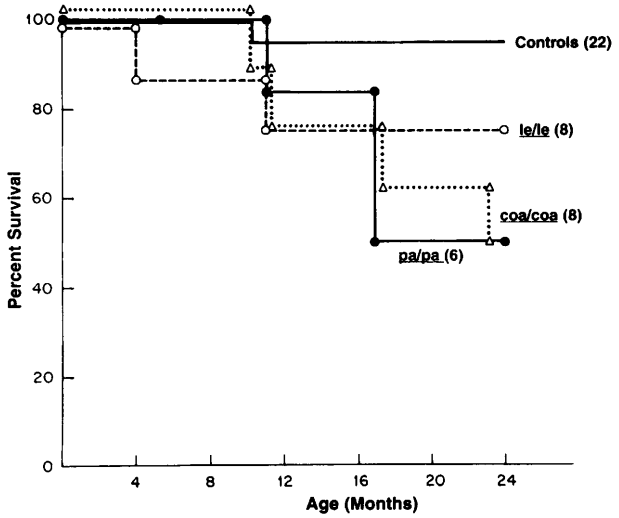

Figure 3. Long-term survival of mutants singly homozygous for the light ear *(le)*, cocoa *(coa)* and pallid *(pa)* genes. *P*-values: *coa/coa* vs. controls (< 0.003); *pa/pa* vs. controls (< 0.0064).

are illustrated. The architecture of the lungs of 2-year-old C57BL/6 J (Fig. 4A) mice did not differ significantly from that of 2-month-old C57BL/6 J animals (not shown). In contrast, the light ear mutant (Fig. 4B) exhibited a particularly striking honeycomb structure with air space enlargement. A similarly severe effect was noted in the pale ear-ruby eye double mutant (Fig. 4C). A similar, though more patchy and less severe derangement of lung architecture was apparent in the pearl mutant (Fig. 4D). Smaller, though still significant, effects were apparent in ruby eye lungs (Fig. 4E). There was no apparent effect of the cocoa or muted mutations (not shown) on lung architecture. No increased infiltrations of macrophages or other leukocytes were apparent in lungs of any mutants compared to control lungs.

A severe honeycomb effect of the beige and pallid mutants (not shown) was also apparent, in agreement with published observations (15). Whereas similarly severe effects on lung pathology were noted in the case of the gunmetal mutant and the muted-pearl double mutant (not shown), it is unfortunately not possible to make definitive statements because lung sections from only the single surviving mutant mice were available.

Several significant autopsy results were noted in individual mutant strains. Lung hemorrhage was apparent macroscopically in four of eight ruby eye, in three of eight light ear, in three of eight pearl and in one of eight cocoa. In all cases, the lung hemorrhages were observed in mutants that had survived the full 2 years of the study. A prolapsed rectum occurred in three of seven beige and in three of five doubly homozygous pale ear-ruby eye. The serious nature of this condition led in part to the sacrifice of these mice prior to the 2-year termination of the experiment. Four of

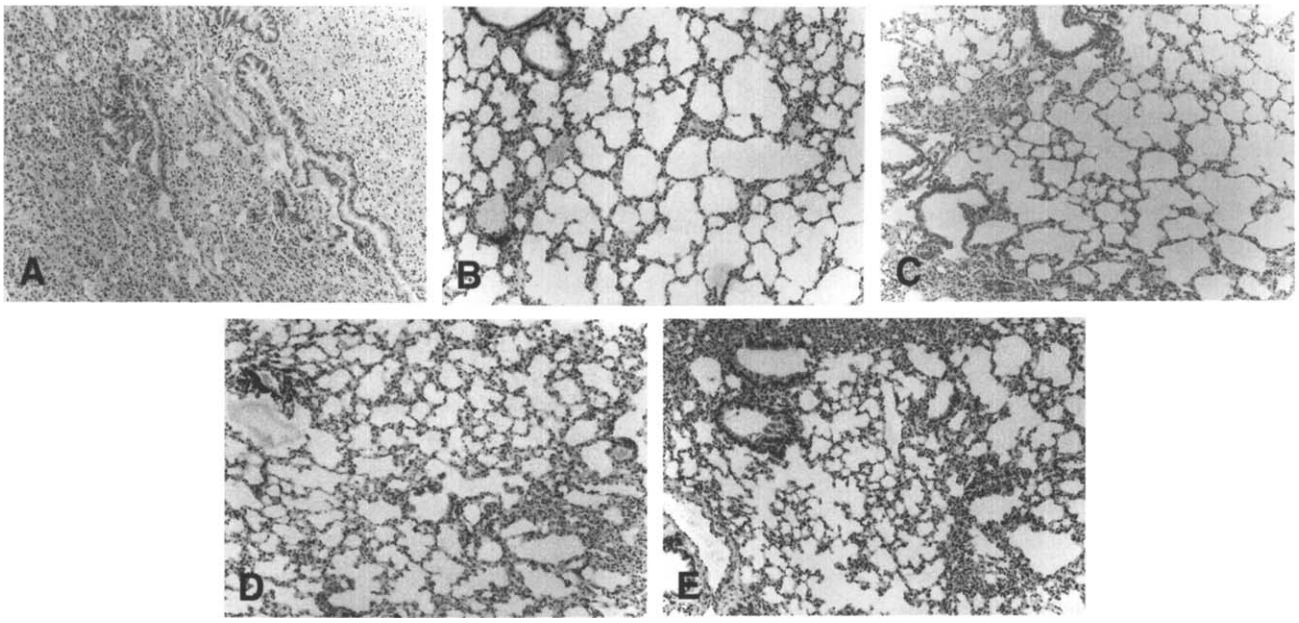

Figure 4. Representative histologic sections from lungs of normal and mutant HPS mice. All sections were taken from 2-year-old mice except for the pale ear-ruby eye double mutants that were 22 months old at the time of sectioning. (A) Normal C57BL/6 J control. (B) light ear. (C) pale ear-ruby eye double mutant. (D) pearl. (E) ruby eye. Hematoxylin-eosin stain. 100×.

five muted mutants had lung tumors. However, the presence of similar lung tumors in two of seven heterozygous *mu/+* control animals suggests these tumors are a product of other genes in this stock background rather than the muted gene.

Mice found dead during the 2-year survival study were subjected to macroscopic external and internal examination. However, no obvious pathology or apparent cause of death could be determined.

Microscopic examination of smears of peripheral blood taken throughout the 2-year study indicated no generalized leukemic or other abnormalities in HPS mutant groups when compared with control blood. Episodal increases in nucleated white cell numbers and occasional monocytoses occurred in several mutant mice. Monocytosis was prevalent in the light ear and pearl mutants. Control C57BL/6 J mice maintained typical differential white cell composition and red cell morphology throughout. One female beige mouse had severe leukemia at 23 months of age. Another female beige mouse within this group had early indications of similar progression.

Discussion

In most cases, mutant HPS-like mouse genes had deleterious effects on long-term survival when mutant mice were compared to congenic control mice. This was especially apparent in the two cases of mice doubly homozygous for two mutant genes, either muted and pearl or pale ear and ruby eye. None of these mice survived the full 2 years of this study. However, decreased survival of stocks homozygous for single mutations was likewise noted. Only a few gunmetal or beige mutants survived 2 years. Intermediate survival times of most other single-gene HPS-like mutants including light ear, pearl, cocoa, and pallid were observed. The muted and ruby eye mutants are apparently quite hardy with almost all surviving the full 2 years of the study. It is doubtful that a more extended study would show effects of these genes on survival, since survival of control C57BL/6 J mice beyond 2 years is limited (16). To our knowledge, there have been no studies of long-term survival of any of the mutants analyzed here. It is noteworthy that, in most cases, altered survival of mutant mice can be attributed to effects of the mutant gene, rather than to unrelated background genes, since the mutant mice are maintained as congenic strains on the C57BL/6 J inbred background.

The reduced life spans observed in the hypopigmentation mutants are consistent with clinical observations of corresponding human patients. CHS patients typically have greatly reduced life spans, often dying in their teen years as a result of repeated infections (5). HPS patients likewise have attenuated life spans. They often die in the fourth to fifth decades of life as a result of fibrotic restrictive lung disease, hemorrhage and colitis, directly caused by the syndrome. In a 5-year study of 242 HPS patients, 47 of 66(71%) died of causes directly related to the syndrome, and of these, 32 deaths (49%) were attributable to lung disease in patients between the ages of 32 and 65 (2). The cause(s) of lung abnormalities in HPS are uncertain.

The reduction in life span of beige mice is consistent with the increased susceptibility to infection and diminished natural killer cell activity observed in this model (5, 12). Gunmetal mice likewise survived poorly. This mutant is known to have severe megakaryocyte/platelet pathologies not found in the other mouse HPS mutants including abnormal membrane complexes in megakaryocytes, thrombocytopenia, and platelets that have morphologically abnormal α granules (17, 18). However, despite these abnormali-

ties, no unusual hemorrhaging was noted in the single necropsied gunmetal mouse.

There are several possible causes of the reduced long-term survival of the other mouse HPS mutants. Diminished natural killer cell activity has been reported in the pale ear and pallid mutants (19). Freedman *et al.* (20) reported defective activation of tumor-killing macrophages in pale ear and light ear. Also, pale ear mice are unable to resolve visceral infections of *Leishmania donovani* (21). Witkop *et al.* (22) sacrificed five pale ear mice at from 100 to 576 days of age and were unable to demonstrate lung or colonic lesions similar to those in HPS patients, though they did note trace accumulation of autofluorescent material in kidney and liver in a 400-day-old animal and a modest amount in a 575-day-old animal.

These histologic studies suggest that lung pathology is present in several of the mouse HPS mutants. Lung pathology, indicated by air space enlargement, was present to varying degrees in most of the mutants. Particularly striking air space enlargements were noted in the light ear, pallid, beige, and double mutant pale ear-ruby eye. Intermediate severity of lung abnormalities occurred in the pearl mutant. At the opposite extreme, minimal or nondetectable effects were apparent in the ruby eye and cocoa mutants. It is noteworthy that, except for the case of the light ear mutant, the severity of lung pathology is inversely proportional to the long-term survival of these various mutants, suggesting that lung pathology may be causal in death, as has been suggested for most human HPS patients (2). The heterogeneity among mouse HPS mutants in development of lung abnormalities has also been observed among HPS patients (23), perhaps reflective of unique molecular etiologies (9–11).

Additional studies are required to determine whether the mouse mutant lung abnormalities are related to the debilitating lung pathology observed in HPS patients. Typically the lung pathology in case reports of human HPS patients has been described as interstitial pulmonary fibrosis (24–26). We have not observed matrix fibers in our lung sections. One possible explanation is that genetically different diseases are being compared. The heterogeneous genetic origins for HPS are well documented in the mouse (3), and recent studies indicate it likewise occurs in humans (9–11). Another possibility is that general species differences between humans and mice produce different pathologies. Thirdly, environmental factors, such as smoking, may interact with genetic determinants in humans to produce lesions different from those observed in mice. On the other hand, it should be noted that there are similarities in the mutant lung abnormalities of the two species. Scattered lung lesions of honeycomb appearance similar to those we have observed in the mouse HPS mutants have been reported in HPS patients (24, 26, 27). Similarly, the lung hemorrhages observed in several of the mouse HPS mutants have been observed in human HPS (23). More detailed studies on both genetically defined human HPS patients and their corresponding appropriate animal models will be required to settle these and related issues.

Lung pathology has been studied in some detail in two of the mouse mutants of this study. Both pallid and beige mice develop spontaneous emphysema with enlargement of air spaces similar to that observed in this study (28). The lungs of beige mice show generalized enlargement of air spaces at all ages whereas lung morphological abnormalities in pallid, including enlargements of air space and destruction of alveolar septa, become apparent only later, at 12 months of age. These results are consistent with our findings of a greater effect of the beige mutation on long-term survival. Pallid mice have an accompanying significant deficiency in serum α 1 proteinase inhibitor, which may be causative of their emphysema-like condition (29). The lung abnormalities observed in beige mice are not commonly reported in CHS patients. A possible explanation is that CHS in humans, unlike beige mice, is usually fatal at very early (teen) years, and lung abnormalities may not have had adequate time for manifestation.

Blood smears were examined in the mouse HPS mutants because of a report (30) of leukemia in the related human platelet storage pool deficiency syndrome. No leukemic abnormalities were apparent in blood of HPS mutants nor have leukemic abnormalities been reported in HPS patients. However, leukemia was observed in two aged beige mice. To our knowledge, leukemia has not been reported previously in this model. This may reflect the fact that mice of this age have rarely been examined. It will be of interest to determine whether there is a relationship between the observation of leukemia and the well-known and often lethal accelerated phase in CHS in which lymphocytes and macrophages actively infiltrate various tissues (31).

Human genes for CHS (32, 33) and for one form of HPS (8, 34) have recently been cloned, and it has been shown that the beige (33, 35) and pale ear (7, 8) mutants are the appropriate animal models for these respective syndromes. The genes are novel with little or no significant homologies to known genes. More recently, two additional mouse HPS mutant genes, mocha (36) and pearl (37), have been identified as encoding the δ and $\beta 3$ A subunits, respectively, of the AP-3 adaptor complex. Therefore, a challenge for the future is to determine mechanisms by which these genes affect long-term survival or lung pathology. High resolution genetic mapping and molecular analyses of other mouse HPS genes are underway in several laboratories. Their successful identification will provide clues to the decreased survival and lung pathologies of this debilitating disease.

Although additional studies on their pathological effects are required, the deleterious influence of these genes on survival and lung structure suggests that several mouse hypopigmentation mutants may serve as appropriate HPS/CHS models for the corresponding human syndromes in future studies of disease mechanisms and therapeutic interventions.

We thank Dr. William Greco for aid with statistical analyses, Debra Tabaczynski for animal care, and Judith Conti for histologic preparation

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