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Necessity of a More Standardized Virological Characterization of Rodents for Aging Studies

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IT is common knowledge that certain murine microorganisms such as viruses have the capacity to cause biological alterations that can have great influence on the course and the results of experiments, and can even lead to premature termination of a study. There is abundant evidence that these agents interfere with a wide diversity of studies, both short- as well as long-term (including aging studies). The loss of breeding production in rodent colonies or loss of the entire colony by overt disease is disruptive to research, and it is relatively easy to persuade an investigator of the usefulness of using virus-free animals after the experience of an overt disease in his colony. However, it is a common characteristic, especially of viral infections in colonies of laboratory animals, that pathogens may persist in a colony without causing any clinical or pathological symptom. This may make results from research less reliable, a point that is frequently overlooked by the investigator. Thus quality biomedical studies of aging are not only dependent on the use of disease-free animals but also on animals free from latent infections.

Hsu (2) and others (1) have summarized much of the data pertaining to the effects of murine microorganisms on biomedical research, and van Zwieten et al. (13) have reviewed the possible influence on aging studies. A great number of the infectious agents reduce the mean lifetime of animals in an infected colony and some will alter such organ systems as the nervous system (1) more or less in their specific functions.

During the past 30 years, progress has been made in identifying and controlling intercurrent diseases and latent infections. Programs to assess the health status of rodents and to characterize their virological status have been developed (2,7). These programs have also been used to monitor animals before they enter the research or breeding colony as well as to control the health status of animals in experiments, in quarantine, and in intramural breeding colonies.

Although in the 1970s it was almost impossible to obtain virus-free animals, during the early 1980s many colonies were caesarian-derived and transferred into barrier type facilities designed to exclude adventitious viruses. Serological data showed that these measures can be effective (12), and many breeding facilities have since adopted them to produce and supply clean animals (5). However, there was only minor improvement in the ability of a great number of institutional and research facilities to maintain laboratory animals free from viruses. Many investigators still ignored the numerous reports describing experimental complications associated with murine infections and continued to fail to appreciate the value of health surveillance testing and barrier maintenance as methods of preventing research variability due to microbiological agents.

More recently, scientists have become increasingly aware of the need for well-standardized laboratory animals. This can be seen in several areas. (A) More rodents are being used in long-term experiments in the field of aging, carcinogenesis, toxicology, nutrition, immunology, and virology. Losses in such studies by intercurrent infection in animals have great financial as well as scientific consequences. (B) In the past much experimental use of laboratory animals was based on endpoint assessments such as mortality and morbidity. Today, we are measuring more subtle changes in specific functions of specialized rodent cells or rodent organ systems. (C) New legislation for controlling the safety of biological products with respect to the hazards of viral contaminants has been introduced by government agencies. There is an increasing application of preparations derived from laboratory animals for diagnostic and therapeutic use in humans. (D) Opposition from antivivisectionists has forced leaders of laboratory animal science to attend carefully to issues of animal protection in experiments—for example, increasing the quality standard of animals reduces the number of animals that are necessary for experiments by reducing the number of experimental failures due to interference with murine infections.

In summary, new standards for quality of animals are being set up. Quality assurance of these animals begins with defining and controlling microbiological status. Thus there is an increasing demand to standardize health monitoring programs and mi-
concerning standardization of the characterization of murine viral paper, and programs for diagnosis of viruses, bacteria, chlamy-

reader is directed. Complete details of microbiological charac-
terization program, and that efforts for such standardiza-
tion are needed. It should first be emphasized that characteriza-
tion of the microbiological status of laboratory rodents is only one facet of a quality assurance program, and that efforts for such standardiza-
tion should be in good relation to other factors such as transporta-
tion systems, quarantine, and housing facilities of the user. When animals are transported under poor conditions and intro-
duced after an inadequate quarantine period in established colo-
yes, there is a high risk of mutual contamination of the animals. Management and the establishment of a full quality assurance program have been covered in a recent review (8) to which the reader is directed. Complete details of microbiological charac-
terization of laboratory animals are also beyond the scope of this paper, and programs for diagnosis of viruses, bacteria, chlamy-
dia, fungi, mycoplasmas, and parasites are readily available (4,7). The purpose of this report is to address basic matters con-
cerning standardization of the characterization of murine viral status.

Viruses of Laboratory Rodents

At the present time more than 25 distinctive virus species
have been isolated from laboratory rodents and their biology and pathogenesis has been described extensively (1). There is, how-
ever, a scarcity of recent reports on the prevalence of viruses, especially in rodents other than mice and rats, and there are also some discrepancies in the selection of viruses used in the screening programs and in the incidences of virus infections reported, with a broad range in prevalence rates (Tables 1 and 2). This divergence may be explained by the fact that different techniques were used in the various screening programs and the results were obtained with colonies from different areas of the world. Fur-
ther, the distribution of some viruses throughout the world (EDIM, cytomegalovirus, thymic virus) is currently unknown and there has been no recent survey of the incidence of LDV infections among laboratory mice.

According to recent reports in Western Europe and North America, the most prevalent viruses in mice are MHV, MVM, sendai, PVM, Reo, and TEMV. In rats the incidence is the highest for the parvoviruses, TMEV, PVM, RCV/SDA, sendai, and Reo. More regular reports on the prevalence of these vi-
ruses not only in murine but also of viruses in other rodents would certainly help in developing a more standardized list of viruses used in monitoring programs.

Methods for Virological Characterization of Laboratory Rodents

Viral infections can be detected by a number of methods which are more or less suitable for routine use:
1. Clinical Signs. As mentioned before, viral infections are often subclinical, so observation of clinical signs is not a reli-
able method; morphological changes in organs are primarily ob-
served in animals that are already seriously ill. Such changes may also not be specific, so pathology has limited value.
2. Virus Isolation. Virus isolation, often termed the “gold standard” to which all other methods should be compared, re-
sults in the identification of the etiological agent. Unsuspected viruses may also be detected. This method, however, requires specialized facilities and is time consuming and expensive. Fur-
thermore, the active phase of infection is often short-lived and may give false negative results. The same holds, in addition, for other methods for virus detection by direct assays for viral anti-
gens or nucleic acid in tissue specimens.
3. Serological Tests. The most generally used method to characterize the viral status of rodents is the demonstration of virus specific immunity by detection of specific antibody with serological tests. These antibodies are specific and persistent and can be rapidly and reliably detected with simple inexpensive techniques. The major disadvantages of the serological test are that unknown viruses will not be detected and for certain viruses there is no serological test available (e.g., lactic dehydrogenase elevating virus, LDV). In some cases of viral infection, no antibody is produced (e.g., lymphocytic choriomeningitis virus, LCM). Since antibody may not appear in serum until 7–14 days after the beginning of infection, serological tests may not be suitable for detection of acute infection.

Another factor that is important for the interpretation of sero-
logical results is potential cross-reactivity. Different strains of viruses may cross-react in a serological test and so the test will not reliably distinguish between individual serotypes [i.e., ELISA (enzyme-linked immunosorbent assay) or FA (fluorescent anti-
tbody test) and parvoviruses] (10).

During the last decade there has been a change in the meth-

| Virus | Mouse hepatitis virus | Sendai virus | Pneumonia virus of mice | Minute virus of mice | Reovirus Type 3 | Theiler’s encephalomyelitis virus | Mouse adenovirus | Polymyositis of mice | Lactic dehydrogenase elevating virus | K virus | Lymphocytic choriomeningitis virus | Lymphocytic choriomeningitis virus | Mouse cytomegalovirus | Thymic virus |
|-------|-----------------------|-------------|------------------------|---------------------|---------------|---------------------------------|----------------|------------------|---------------------------------|---------|----------------------------------|---------------------------------|----------------|---------------------|
| ND: Only limited data available. | 49% (29–73%) | 32% (7–57%) | 32% (3–83%) | 22% (2–21%) | 24% (0–52%) | 21% (7–40%) | 2% (0–8%) | 4% (0–14%) | 5% (0–14%) | 5% (0–33%) | 0% | ND | ND | ND |

| Virus | Sendai virus | Pneumonia virus of mice | Kilham rat virus | Reovirus 3 | Adenovirus | Tooldan H-1 | Theiler’s encephalomyelitis virus | Rat cytomegalovirus | Hantaan virus | Rat rotavirus |
|-------|-------------|------------------------|-----------------|-----------|------------|-------------|---------------------------------|-----------------|-------------|-----------|
| ND: Only limited data available. | 41% (16–75%) | 34% (10–55%) | 41% (16–70%) | 39% (21–74%) | 19% (0–44%) | 7% (0–17%) | 31% (4–71%) | ND | ND | ND |
ods used in serology. Traditional tests such as CF (complement fixation) are disappearing and new techniques have been introduced. These different test systems have been reviewed extensively (9). Although the insensitive CF is still sometimes used, it is well accepted now that ELISA and FA are the primary techniques of choice and that HAI (hemagglutination inhibition) may sometimes be used as an alternative method to confirm primary test results or to specify strains. The ELISA is technically easy to perform and is more sensitive than CF and HAI. FA is more expensive and time consuming and slides must be interpreted by trained experienced personnel in order to obtain valid results. Furthermore, although FA is less quantitative it is especially suited as a confirmatory assay and should be used in the event of positive results. One of the major problems of ELISA (and FA) is the lack of a standard method for performing the test or reporting the results among different laboratories.

Another new test, the Western Blotting technique (11), identifies the viral proteins to which antibody is produced. It is an excellent alternative, and provides reliable confirmation of primary positive test results. Due to costs this technique will probably only be applied in research and diagnostic reference laboratories.

Standard operating procedures for methodology used by testing laboratories and the availability of standardized viral antigens and reference sera (with activities expressed in international units) would be of great help in endeavors to increase the inter-laboratory standardization of the microbiological characterization of rodents.

SCREENING PROFILES

There are a number of factors to consider with regard to which viruses should be included in health monitoring of rodent populations:

1. Is the agent capable of episodically infecting rodents, does the enzootic infection result in clinical or histological diseases, and are rodents the primary host?
2. Does the virus have an established effect on research other than overt clinical signs or death?
3. Is the agent ubiquitous, and is there any hazard to humans such as zoonotic potential of LCM and Hantaan?
4. What are the specific requirements of the investigator?
5. What is the aim of the testing program? Is the screening program for the microbiological characterization of large breeding populations, for more frequent control of defined breeding units (barrier-maintained or not), or for all incoming animals?
6. How significant or common are the viruses that may be encountered? Some viruses are infrequently encountered and therefore do not represent significant problems to breeders or investigators, whereas others are frequently found. In the latter case, limited programs rather than total microbiological analyses may be used for controlling microbiological status during the course of an experiment. Sentinel animals with a defined health status can be used when it is difficult to obtain samples from animals in a study.

In summary, there is no single answer as to how a screening list should be formulated. Different lists have been published, and different lists are in use in the various diagnostic laboratories and breeding facilities. A proposal for the formulation of a more standardized screening list for murine viruses according to our recent knowledge and increasing demands is shown in Tables 3 and 4. Rarely can each shipment of animals from outside sources be checked thoroughly before use. Therefore, reliability and standardization of the health reports from the supplier play a very critical role.

| Virus | Total | Limited |
|-------|-------|---------|
| Sendai virus | X | X |
| Mouse hepatitis virus | X | X |
| Pneumonia virus of mice | X | X |
| Mink virus of mice | X | X |
| Reovirus Type 3 | X | X |
| Theiler’s encephalomyelitis virus | X | X |
| Extrememia virus | X | - |
| Mouse adenovirus | X | - |
| Polyoma virus | X | - |
| K virus | X | - |
| Lymphocytic choriomeningitis virus | X | - |
| Hantaan | X | - |
| Edim | X | - |
| Lactic dehydrogenase virus | X | - |
| Mouse cytomegalovirus | X | - |
| Thymic virus | X | - |

SAMPLE COLLECTION, PROCESSING, AND SHIPMENT

Blood may be obtained aseptically and without additives from the brachial vessels, jugular vein, peri-orbital venous sinus, or heart. Hemolysis should be avoided by not forcing the blood through a small needle or storing the blood too long prior to separation of the serum fraction by centrifugation (for 5 to 10 min at 2000 × g). Serum before testing can be stored at 4°C and should be frozen at −20°C for longer periods of time.

Shipment is possible by express mail either at lowered temperature or after addition of a preservative (0.01% thimerosal). Labeled samples should be accompanied with information on the serum dilution, rodent species, and name and telephone/telex or fax number of the sender.

Pooling of sera should absolutely be avoided as this will reduce antibody titre levels. Indeed, sera may become useless if one of the samples contains nonspecific activity [for more detailed information on serum collection, see (4)].
governed by a number of factors. One sampling reflects the acceptable confidence limit (>0.95). If, for example, an infection of new animals and biological materials, economic type of physical facilities, husbandry practices, rate of introduc-
tual status in a colony, but this may change after sampling. The should be used.
multiple agents are screened, the lowest expected morbidity rate rate of 30% is expected, then 9 samples should be taken. If that will give at least one positive reaction with a normally ac-
cent of infection in a population, and assuming that the screen-
at least 100 or more animals. In short, given an expected per-
sample size. The sample size

| Expected Incidence of Infection | Sample Size |
|---------------------------------|-------------|
| 10                              | 29          |
| 20                              | 14          |
| 30                              | 9           |
| 40                              | 6           |
| 60                              | 4           |
| 80                              | 2           |

SAMPLING SCHEDULES

For purposes of serology retired breeders of immunologically competent strains are generally recommended for monitoring. Sample size is based on statistical considerations which have been discussed elsewhere (2), and formulas have been developed which may be used to calculate the sample size. The sample size is independent of the size of the population if the population is at least 100 or more animals. In short, given an expected percent of infection in a population, and assuming that the screening procedure is 100% accurate, Table 5 shows a sample size that will give at least one positive reaction with a normally acceptable confidence limit (>0.95). If, for example, an infection rate of 30% is expected, then 9 samples should be taken. If multiple agents are screened, the lowest expected morbidity rate should be used.

There is no single answer regarding test frequency, which is governed by a number of factors. One sampling reflects the actual status in a colony, but this may change after sampling. The type of physical facilities, husbandry practices, rate of introduction of new animals and biological materials, economic consider-

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