3

The importance of disease in immunodeficient mice and rats

S. Sparrow

(Medical Research Council Laboratory Animals Centre, Woodmansterne Road,
Carshalton, Surrey SM5 4EF, UK)

INTRODUCTION

The very features that make the immunodeficient animal so valuable in research also present those responsible for breeding and maintaining it with the greatest problems.

Diseases are much more common in these animals than in those with an intact immune system, in fact, if one accepts a common definition of disease, 'a specific state of malfunction of an animal body' (Dorland, 1959) then one must accept that immunodeficient animals are in a permanent diseased state.

This paper will be confined, however, to the more specific aspects of naturally occurring disease with particular emphasis on infectious agents.

VIRAL DISEASE

Mouse Hepatitis Virus

Although it is in the nude mouse (*nu nu*) that the wasting syndrome has attracted most attention, a very similar condition was first described in neonatally thymectomised mice (East *et al.*, 1963). In that outbreak mouse hepatitis virus (MHV) was shown to be involved, causing necrotic foci in the liver.

The pathogenesis of MHV infection is very complex as there are a number of different strains of the virus which may produce quite different pathological effects. Moreover, responses vary with age and strain of mouse.

The first strain of virus identified has since been called MHV-1 (Dick *et al.*, 1956). It produces little evidence of disease in normal adult mice; although there may be a transient interstitial pneumonia after infection via the respiratory route in weanling mice (P. Carthew and S. Sparrow, unpublished observations). In the thymus-deprived mouse, the naturally athymic nude mouse, the mouse made immunodeficient by treatment with antilymphocyte serum or cortisone and in the mouse with intercurrent disease such as *Eperythrozoon coccoides* infection or murine leukaemia it can produce extensive liver lesions as shown in figure 3.1

Figure 3.1 MHV-1 infection in the nude mouse. Extensive liver necrosis and some cellular infiltration. Haematoxylin and eosin, $\times 240$.

(East *et al.*, 1963; Pantelouris, 1968; Allison, 1970; Gallily *et al.*, 1964; Gledhill *et al.*, 1955; Nelson, 1952). In the first two cases, thymus-deprived and nude mice, chronic disease from infection with MHV-1 results in the characteristic 'wasting syndrome'.

A very similar picture of wasting has been described by Fujiwara and his co-workers (1977) with a strain of virus he designated as MHV-U. This virus causes 100 per cent mortality in nude mice but only produces disease in heterozygous litter mates after the injection of cortisone.

Four strains of virus that produce overt disease in normal mice (JHM, MHV-S, MHV-2, MHV-3) produce rapid death in nude mice, as distinct from the wasting syndrome. The pathogenesis of the infection may be different in the immunodeficient animal. In the nude mouse death is invariably associated with extensive liver necrosis, but in euthymic mice the target organ of JHM infection is the brain and spinal cord where it causes demyelination (Cheever, 1949), and MHV-3 is now thought to be responsible for the disease previously known as lethal intestinal virus of infant mice (LIVIM) which is manifest by a fatal diarrhoea in preweaning mice (Broderick *et al.*, 1976; Carthew, 1977).

Another strain, MHV-A59, which can cause acute liver necrosis in normal mice (Manaker *et al.*, 1961) appears from initial investigations to be more chronic in nude mice leading again to a wasting syndrome (P. Carthew and S. Sparrow, unpublished observations).

Sendai Virus

Sendai virus infection can be responsible for extensive losses in both normal mouse and rat colonies (Burek *et al.*, 1977; Zurcher *et al.*, 1977). The lesions are typically necrosis of the bronchial epithelium and subsequent hyperplasia (figure 3.2). Necrotic bronchial epithelial cells and inflammatory cells accumulate in the bronchial lumen (figure 3.3) and there may be an interstitial pneumonia localised around the damaged bronchioles (Carthew and Sparrow, 1979). Frequently, however, the infection is inapparent.

Figure 3.2 Sendai virus infection in the mouse. Bronchiolar hyperplasia. Haematoxylin and eosin $\times 200$.

In mice treated with cyclophosphamide an otherwise mild infection with this virus is converted into a lethal one (Robinson *et al.*, 1968).

In the nude mouse and the nude rat (Festing *et al.*, 1978) the virus and the lesions it produces persist for a considerable time. We have demonstrated, using immunoperoxidase staining of lung sections (figure 3.4), the presence of virus in the bronchial epithelium for at least 35 days (in the case of nude mice) and 32 days (in the case of nude rats) post infection, compared with only 9 days in immuno-competent mice and rats (table 3.1).

A number of other workers have also shown a chronic infection with this virus in nude mice which has resulted in a wasting syndrome with clinical features very similar to those produced by MHV (Gershwin *et al.*, 1975; Ward *et al.*, 1976; Ueda *et al.*, 1977).

Figure 3.3 Sendai virus infection in the mouse. The formation of bronchial plugs and peribronchial interstitial pneumonia. Haematoxylin and eosin, $\times 320$.

Figure 3.4 Sendai virus infection in the mouse. Immunoperoxidase staining shows the presence of virus in the bronchial epithelium. Counterstained with haematoxylin, $\times 160$.

Table 3.1 Sendai virus (para-influenza type I) in AGUS rats (solid arrows) and rnu rnu rats (dashed arrows).

Days	Peroxidase staining	Epithelial stripping	Epithelial hyperplasia	Bronchial plugs	Interstitial pneumonitis	Alveolar collapse
1						
2						
3	↕	↕				
4	↕	↕	↕	↕		
5	↕	↕	↕	↕	↕	
6	↕	↕	↕	↕	↕	
7	↕	↕	↕	↕	↕	↕
8	↕	↕	↕	↕	↕	↕
9	↕	↕	↕	↕	↕	↕
10	↕	↕	↕	↕	↕	↕
12	↕	↕	↕	↕	↕	↕
14	↕	↕	↕	↕	↕	↕
16	↕	↕	↕	↕	↕	↕
18	↕	↕	↕	↕	↕	↕
20	↕	↕	↕	↕	↕	↕
23	↕	↕	↕	↕	↕	↕
32	↕	↕	↕	↕	↕	↕

Pneumonia Virus of Mice

In complete contrast to Sendai virus another important murine respiratory virus, pneumonia virus of mice (PVM) which also causes necrosis and hyperplasia of the bronchial epithelium, does not persist in the lungs of nude mice. The very exten-

Figure 3.5 PVM infection in the mouse lung. Severe interstitial pneumonia. Haematoxylin and eosin, $\times 200$.

sive interstitial pneumonia (figure 3.5) that characterises this infection (Carthew and Sparrow, 1979) is neither more severe nor lasts for any greater length of time than in the euthymic animals. It is relevant to note that unlike most other naturally occurring rodent viruses PVM does evoke an antibody response in nude mice and this is possibly an IgM response.

Other Murine Viruses

Early clinical evidence suggests that Parker's rat corona-virus (PRCV) which causes tracheitis and interstitial pneumonia (Bhatt and Jacoby, 1977) of short duration in normal rats may give a persistent low grade interstitial pneumonia in nude rats (figure 3.6). Sialodacryoadenitis virus (SDA), which is closely related antigenically

Figure 3.6 PRCV infection in the rat lung. Mild interstitial pneumonia. Haematoxylin and eosin, $\times 280$.

to PRCV, causes necrosis of the intercalated and striated ducts of the salivary gland (figure 3.7) and necrosis with cellular infiltration of the Harderian gland (figure 3.8) is very widespread in rat colonies in the United Kingdom. There is again clinical evidence in the form of periorbital porphyria and corneal ulceration that this disease may be more severe in nude rat colonies. The so called masked rat (Kent *et al.*, 1976) has an appearance very similar to a nude rat infected with SDA and there is certainly some suggestion that this mutant is more susceptible to infection and therefore perhaps immunodeficient (Festing, 1979).

Another rat virus that is very widespread and which may prove to be impor-

Figure 3.7 SDA infection in the rat sub-maxillary salivary gland. Necrosis of the striated ducts. Haematoxylin and eosin, $\times 280$.

Figure 3.8 SDA infection in the rat Harderian gland. Necrosis of glandular tissue with cellular infiltration. Haematoxylin and eosin, $\times 280$.

tant in nude rats is Kilham rat virus. This parvovirus has a predisposition for replicating cells, it can be transplacentally transmitted and often exhibits its effects in the foetus causing malformations and runting.

There is no evidence that other murine viruses known to cause problems in the United Kingdom (Carthew *et al.*, 1978) have any particular significance for immunodeficient animals. There are, however, two other points that should be emphasised with regard to viral infections.

First, the general lack of antibody response in immunodeficient animals means that normal serological diagnosis in these animals is not possible, diagnosis must therefore be confirmed by viral isolation, the mouse antibody production test (MAP) or the serological examination of heterozygous litter mates.

Second, the great value of immunodeficient animals for xenografting and allo-grafting procedures must be measured against the very real danger of passively transmitting viral disease from one colony to another or even one country to another by means of tumour transplants.

A summary of the viral diseases as they may affect immunodeficient animals is given in table 3.2.

Table 3.2 Viral pathogenicity

Species	Virus	Normal	Immunodeficient
Mice	MHV-1	—	+++
	MHV-2	+++	+++
	MHV-3	+++	+++
	MHV-JHM	++	+++
	MHV-S	++	+++
	MHV-A59	++	+++
	MHV-U	—	+++
	Sendai	+	+++
	PVM	+	+
	Reo III	+	?
	EDIM	+	?
Rats	Sendai	+	+++
	PVM	+	?
	Coronaviruses	++	?
	Kilham rat virus	+	?

BACTERIAL DISEASE

Very high losses due to septicaemia have been reported in mice subjected to whole body irradiation (Stoner *et al.*, 1965). The organism responsible, *Pseudomonas aeruginosa*, is an ubiquitous organism that thrives in moist conditions. It can also be frequently isolated as the sole organism from preputial glands and subcutaneous abscesses in nude mice. An even more common cause of abscesses in these animals

is *Pasteurella pneumotropica*. Orbital abscesses can be particularly troublesome (figure 3.9), those caused by *P. pneumotropica* do respond to antibiotic therapy (Moore and Aldred, 1978). *Klebsiella* spp. have also been implicated in eye infections and the lack of eyelashes and consequent contamination of the conjunctival sac with particulate material may be significant in these cases (McGary *et al.*, 1976). Conjunctivitis in the nude rat due to *P. pneumotropica* has also been reported (Moore, 1979). Problems arising from *Staphylococcus aureus* infection of the skin

Figure 3.9 Periorbital abscess and corneal ulceration in the nude mouse infected with *P. pneumotropica*.

in nude mice are relatively uncommon. Bywater (1977) described a case of furunculosis due to this organism. We have seen, however, a large number of nude rats with multiple skin ulcerations (figure 3.10) which were heavily contaminated with *S. aureus*.

Tyzzer's disease (*Bacillus piliformis* infection) can reach epizootic proportions in normal rodent colonies but often occurs as a result of some form of stress. The immunological status of the animal does have considerable importance; a standard method of detecting latent contamination in a colony is to inject animals with cortisone to precipitate the clinical disease, and thymus-deprived mice are particularly susceptible to infection. Another interesting feature is that whereas in normal rats the target organs are usually the intestine and the heart (figure 3.11), in an outbreak in our own nude rat colony the salient feature was extensive liver necrosis (figure 3.12), which is characteristic of the disease in most other species. Also in this outbreak the heterozygous-normal litter mates were unaffected.

Figure 3.10 Multiple ulceration of the skin of the nude rat with heavy *S. aureus* contamination.

Figure 3.11 The organisms of Tyzzer's disease (*Bacillus piliformis*) in the myocardium of the rat. $\times 640$.

Figure 3.12 Multiple necrotic foci in the liver of a nude rat infected with *B. piliformis*.

PARASITES

Among the commonest contaminants of barriered rodent colonies are intestinal protozoa (Sparrow, 1977). Boorman *et al.* (1973) have recorded the severe effects that *Hexamita muris* and *Giardia muris* can have on nude mice and it is possible that other flagellates are also pathogenic in immunodeficient animals.

The failure of nude mice to control the pinworms *Aspicularis tetraptera* and *Syphacia obvelata* (Jacobson and Reed, 1974) and the cestode *Hymenolepis nana* (Reed *et al.*, 1977) would suggest that infection with these relatively non-pathogenic metazoan parasites could present problems in nude mouse colonies.

NON-INFECTIOUS DISEASE

The nude mouse is not truly hairless; it has large numbers of hair follicles. The hairs that are produced, however, are abnormally keratinised and generally break off at the skin surface or fail to penetrate the keratin layer of the skin in a normal fashion. This feature coupled with the high frequency of hair growth cycles (Eaton, 1977) can give rise to some unusual effects. A thick layer of keratin forms at the skin surface and may be shed as flakes or in more extreme cases as large plaques which become particularly evident in pigmented strains due to a build up of melanin granules in this layer (figure 3.13). The nude rat hairs appear similar to those of nude mice; hair follicles are not as numerous as in mice but are comparable with those in normally haired rats. The hairs are again abnormally keratinised and break off at the skin surface (figure 3.14).

Figure 3.13 Nude mouse skin showing keratin plaque formation heavily loaded with melanin. Haematoxylin and eosin, $\times 200$.

Two aged nude rats (over one year old) so far examined have both shown glomerular nephritis with tubular dilation and hyaline casts (figure 3.15). It is, however, known that some otherwise normal strains of rats have a high incidence of glomerular nephritis at this age and it is quite possible that the findings in the nude rats are a function of the background genetic make up rather than the nude mutation itself.

NEOPLASIA

Much has been written about oncogenesis in the nude mouse (see Rygaard and Povlsen, 1974a; Nomura *et al.*, 1977) and it is this animal that has been at least partly responsible for the swing in popularity from immunosurveillance theories to immunostimulation theories of carcinogenesis. Since the finding of high levels of cytotoxic effector cells in nude mice the immunosurveillance theory seems to have gained ground again but broadened to incorporate the function of natural killer cells (NKC).

Early workers reporting a total absence of 'spontaneous' tumours in nude mice were using for their observations mice with an average lifespan of less than 4 months and maximum of 7 months (Rygaard and Povlsen, 1974b). The first lymphoreticular tumour was reported in a 5-month-old nude mouse (Custer *et al.*, 1973) and since that time other workers have reported a relatively high incidence of lymphoreticular tumours in nude mice (Outzen *et al.*, 1975).

Figure 3.14 Nude rat skin. Abnormally keratinised hair shafts near skin surface. Haematoxylin and eosin, $\times 280$.

There are still, however, few reports of spontaneous solid tumours in nude mice. The apparent bias toward lymphoreticular tumours is not unique to the nude mouse. Sacksteder (1976) in comparing spontaneous tumour incidence in germ-free and conventional rats found that the incidence of lymphoreticular tumours was essentially the same but that there were significantly fewer solid tumours in the germ-free group. Since immunodeficient animals reach the 'normal' age of tumourigenesis only under germ-free, or at least high barrier, conditions, it may be that freedom from disease rather than immunological status *per se* influences tumour incidence.

One example will illustrate this point. The proliferative and metaplastic changes in the bronchial epithelium associated with Sendai virus infection in nude mice may be so marked as to resemble adenomas (Sharkey, 1978). Figure 3.16 shows a section of lung from a thymus-deprived mouse that was shown by immunoperoxidase staining to be infected with Sendai virus; the proliferative lesion in the

Figure 3.15 Glomerular nephritis in the nude rat. Haematoxylin and eosin, $\times 240$.

Figure 3.16 Proliferative lesion in the lung parenchyma of a thymus-deprived mouse infected with Sendai virus. Haematoxylin and eosin, $\times 320$.

lung parenchyma has a fair number of mitotic figures, cells show a striking similarity to the hyperplastic cells of the bronchial epithelium and resembles an adenoma. Is it possible, therefore, that in trying to protect our immunodeficient animals from the devastating effects of infectious disease we are also protecting them from neoplasia?

REFERENCES

- Allison, A. C. (1970). Effects of antilymphocytic serum on bacterial and viral infections and virus oncogenesis. *Fedn Proc. Fedn Am. Socs exp. Biol.*, 29, 167-8
- Bhatt, P. N. and Jacoby, R. O. (1977). Experimental infection of adult axenic rats with Parker's rat coronavirus. *Arch. Virol.*, 54, 345-52
- Boorman, G. A., Lina, P. H. C., Zurcher, C. and Nieuwerkerk, H. T. M. (1973). *Hexamita and Giardia* as a cause of mortality in congenitally thymus-less (nude) mice. *Clin. exp. Immunol.*, 15, 623-7
- Broderson, J. R., Murphy, F. A. Hierholzer, J. C. (1976). Lethal enteritis in infant mice caused by mouse hepatitis virus. *Lab. Animal Sci.*, 26, 824
- Burek, J. D., Zurcher, M. C., van Nunen, M. C. J. and Hollander, C. F. (1977). A naturally occurring epizootic caused by Sendai virus in breeding and aging rodent colonies. II. Infection in the rat. *Lab. Animal Sci.*, 27, 963-71
- Bywater, J. E. C. (1977). Furunculosis in a mouse. *Vet. Rec.*, 101, 385
- Carthew, P. (1977). Lethal intestinal virus of infant mice is mouse hepatitis virus. *Vet. Rec.*, 101, 465
- Carthew, P. and Sparrow, S. (1979). A comparison of the pathogenesis of Sendai virus and pneumonia virus of mice infections in germfree mice using the immunoperoxidase technique on paraffin sections. *J. Path.*, in press
- Carthew, P., Sparrow, S. and Verstraete, A. P. (1978). Incidence of natural virus infections of laboratory animals 1976-1977. *Lab. Animals*, 12, 245-6
- Cheever, F. S. (1949). A murine virus (JHM) causing disseminating encephalomyelitis with extensive destruction of myelin. *J. exp. Med.*, 90, 181-94
- Custer, R. P., Putzen, H. C., Eaton, G. J. and Prehn, R. T. (1973). First recorded spontaneous lymphoma in a 'nude' mouse. *J. natn. Cancer Inst.*, 51, 707-11
- Dorland, W. A. N. (1959). *Dorland's Pocket Medical Dictionary*, W. B. Sanders, Philadelphia
- Dick, G. W. A., Niven, J. S. F. and Gledhill, A. W. (1956). A virus related to that causing hepatitis in mice (MHV). *Br. J. exp. Path.*, 37, 90-8
- East, J., Parrot, D. M. V., Chesterman, M. B. and Pomerance, A. (1963). The appearance of a hepatotropic virus in mice thymectomised at birth. *J. exp. Med.*, 118, 1069-82
- Eaton, G. J. (1977). Hair growth waves and cycles in nude mice. In *Proceedings of the Second International Workshop on Nude Mice* (T. Nomura, N. Ohsawa, N. Tamaoki and K. Fujiwara eds), University of Tokyo Press, Tokyo and Gustav Fischer Verlag, Stuttgart, pp. 89-93
- Festing, M. F. W. (1979). Inherited immunological defects in laboratory animals. In this volume, chapter 2
- Festing, M. F. W., May, D., Connors, T. A., Lovell, D. and Sparrow, S. (1978). An athymic nude mutation in the rat. *Nature, Lond.*, 274, 365-6
- Fujiwara, K., Tamura, T., Taguchi, F., Hriano, N. and Ueda, K. (1977). Wasting disease in nude mice infected with facultatively virulent mouse hepatitis virus. In *Proceedings of the Second International Workshop on Nude Mice* (T. Nomura, N. Ohsawa, N. Tamaoki and K. Fujiwara, eds), University of Tokyo Press, Tokyo and Gustav Fischer Verlag, Stuttgart, pp. 53-60

- Gallily, R., Wanwick, A. and Bang, F. B. (1964). Effect of cortisone on genetic resistance to mouse hepatitis virus *in vivo* and *in vitro*. *Proc. natn. Acad. Sci. U.S.A.*, **51**, 1158-64
- Gershwin, M. E., Merchant, B., Gelfand, M. C., Vickers, J., Steinberg, A. D. and Hansen, C. T. (1975). The natural history and immunopathology of outbred athymic (nude) mice. *Clin. Immunol.*, **4**, 324-40
- Gledhill, A. W., Andrews, C. H. and Dick, G. W. A. (1955). The nature of mouse hepatitis virus in weanling mice. *J. Path. Bact.*, **69**, 311
- Jacobson, R. H. and Reed, N. D. (1974). The thymus dependency of resistance to pinworm infection in mice. *J. Parasitol.*, **60**, 976
- Kent, R. L., Lutzner, M. A. and Hansen, C. T. (1976). The masked rat: an X-ray induced mutant with chronic blepharitis, alopecia and pasteurellosis. *J. Hered.*, **67**, 3-5
- McGarry, M. P., Bahny, L., Jividen, J., Williams, P., Mirand, E. A. and Murphy, G. P. (1976). Particulate matter induced periorbital abscess in gnotobiotic athymic n^u/n^u (nude) mice. *Lab. Animal Sci.*, **26**, 956-8
- Manaker, R. A., Piczak, C. V., Miller, A. A., Stanton, M. F. (1961). A hepatitis virus complicating studies with mouse leukaemia. *J. natn. Cancer Inst.*, **27**, 29-51
- Moore, G. J. (1979). Conjunctivitis in the nude rat (rnu/rnu). *Lab. Animals*, **13**, 35
- Moore, G. J. and Aldred, P. (1978). Treatment of *Pasteurella pneumotropica* abscesses in nude mice (nu/nu). *Lab. Animals*, **12**, 227-8
- Nelson, J. B. (1952). Acute hepatitis associated with mouse leukaemia. I. Pathological features and transmission of the disease. *J. exp. Med.*, **96**, 293-302.
- Nomura, T., Ohsawa, N., Tamaoki, N. and Fujiwara, K. (eds) (1977). *Proceedings of the Second International Workshop on Nude Mice*, University of Tokyo Press, Tokyo and Gustav Fischer Verlag, Stuttgart
- Outzen, H. C., Custer, R. P., Eaton, G. H. and Prehn, R. T. (1975). Spontaneous and induced tumour incidence in germ-free 'nude' mice. *J. reticuloendothel. Soc.*, **17**, 1-9
- Pantelouris, E. M. (1968). Absence of thymus in nude mouse mutant. *Nature, Lond.*, **217**, 370-1
- Reed, N. D., Isaak, D. D. and Jacobson, R. H. (1977). The use of nude mice in model systems for studies on acquired immunity to parasitic infections. In *Proceedings of the Second International Workshop on Nude Mice* (T. Nomura, N. Ohsawa, N. Tamaoki and K. Fujiwara, eds), University of Tokyo Press, Tokyo and Gustav Fischer Verlag, Stuttgart, pp. 3-16
- Robinson, T. W. E., Cureton, R. J. R. and Heath, R. B. (1968). The effect of cyclophosphamide on Sendai virus infection of mice. *J. med. Microbiol.*, **2**, 137-45
- Rygaard, J. and Povlsen, C. O. (eds) (1974a). *Proceedings of the First International Workshop on Nude Mice*, Gustav Fischer Verlag, Stuttgart
- Rygaard, J. and Povlsen, C. O. (1974b). The nude mouse does not develop spontaneous tumours. *Acta path. microbiol. scand. B*, **82**, 99-106
- Sacksteder, W. H. (1976). Spontaneous tumours in the germ-free F 344 rat. *J. natn. Cancer Inst.*, **57**, 1371-3
- Sharkey, F. E. (1978). Histopathological observations on a nude mouse colony. In *The Nude Mouse in Experimental and Clinical Research* (J. Fogh and B. C. Giovanella, eds), Academic Press, New York, pp. 75-93
- Sparrow, S. (1977). Causes of microbiological contamination in commercial breeding units. *Z. Versuchstierk. Bd*, **19**, 210-5
- Stoner, R. D., Hess, M. W. and Bond, V. P. (1965). *Radiation and Infection: An Annotated Bibliography*, Medical Research Center, Brookhaven National Laboratory, Upton, N.Y.
- Ueda, K., Tamura, T., Machii, K. and Fujiwara, K. (1977). An outbreak of Sendai virus infection in a nude mouse colony. In *Proceedings of the Second International Workshop on Nude Mice* (T. Nomura, N. Ohsawa, N. Tamaoki and K. Fujiwara, eds), University of Tokyo Press, Tokyo and Gustav Fischer Verlag, Stuttgart, pp. 61-69

- Ward, J. W., Houchens, D. P., Collins, M. J., Young, D. M. and Reagan, R. L. (1976). Naturally occurring Sendai virus infection of athymic nude mice. *Vet. Path.*, 13, 36-46
- Zurcher, C., Burek, J. D., van Nunen, M. C. J. and Hollander, C. F. (1977). A naturally occurring epizootic caused by Sendai virus in breeding and aging rodent colonies. I. Infection in the mouse. *Lab. Animal Sci.*, 27, 955-62