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Letter to the Editor

Differences in animal housing facilities and diet may affect study outcomes—a plea for inclusion of such information in publications

No journal would accept a manuscript about microbiology or tissue culture without information on the medium or growth conditions used. However, manuscripts about transgenic animals infrequently mention the animal housing facilities and almost never mention the diet used. Since the publication volume of manuscripts dealing with transgenics increases dramatically and as discussed below there is emerging evidence that the housing facilities and the diet may have an effect on the phenotypes observed we urge inclusion of this information in manuscripts dealing with transgenics.

It is not infrequent for researchers in different laboratories and sometimes even within a single laboratory to observe different phenotypes on the same transgenic animals. Here, we attempt to address the issue of the life span of the same knock-out mice being significantly different from one study to the next. To exemplify this phenomenon, we compiled data for *Atm* deficient mice used as animal model for the cancer-prone disorder ataxia telangiectasia (AT). Previous studies showed that $Atm^{-/-}$ mice die of thymic lymphomas between ages of 2 and 5 months [1–3]. In our study, at 5 months, 94% of $Atm^{-/-}$ mice were alive and 50% still lived after 12.5 months (unpublished data). During our ongoing study other investigators also reported that survival of *Atm* deficient mice is much longer than in earlier studies. For example, 50% mice were alive at 7 [4–6] or 10 months of age [7] and 40% $Atm^{-/-}$ mice still lived after 18 months [4].

Different animal backgrounds may often account for mixed study outcome. However, survival studies on *Atm* deficient mice have failed to show any differences between inbred and mixed backgrounds. Median survival of 129SvEv background mice varied from 2.5 to 7.5 months [6,8–10] and the median survival of 129SvEv:C57BL/6J mice was 4–10 months [4,5,7,10] (Table 1). Thus, there seem to be other factors affecting tumor onset and mouse survival.

As far back as the 1960s researchers observed that improved environmental conditions during the 1960s as compared to the 1940s and 1950s increased the lifespan of inbred mice [11,12]. Reduced levels of pathogens and improved diets were considered important factors contributing to prolonged lifespan.

1. Specific-pathogen-free (SPF) versus non-SPF animal facilities

Specific-pathogen-free (SPF) animals that have been defined as 'animals that are free of specified micro-organisms and parasites, but not necessarily free of others not specified' (International Committee on Laboratory Animals, 1964) are usually devoid of all demonstrable pathogenic micro-organisms. Researchers in the AT field have proposed that *Atm* deficient mice live longer when housed in SPF facilities [9,10]. This seemed as an attractive explanation and we intended to elaborate on this further by comparing survival of *Atm* deficient mice in SPF versus non-SPF facilities from published studies. However, only 3 out of 12 publications have indicated the nature of the animal room (Table 1). Only in one article [7], authors included this information in materials and methods section, while in other two papers, it was put in the results [9] and discussion [10] sections. Comparison of survival data where information on the nature of animal facility is provided in the publication (in total four studies including our own unpublished data) shows that *Atm* deficient mice in SPF facilities live twice as long as in non-SPF animal rooms (median survival 10–12 months versus 4–5 months). Most studies summarized in Table 1 used the same knock-out $Atm^{-/-}$ mice generated by Barlow et al. [2]. Thus, the nature of animal facility might modulate study outcome. This suggests that providing information on animal housing facility (SPF versus non-SPF) in the manuscripts' materials and method section may be helpful to understand some differences in survival of transgenic mice. In no way, do we suggest that pathogen status in the facility is the cause for differences in phenotype. Phenotypic differences have been found in different institutions

Table 1 – Medial survival of *Atm* deficient mice in different studies

50% survival (month)	Background	Animal facility	Reference	Mice generated by
2.2	129SvEv, Black Swiss, 129SvEv:Black Swiss	Not indicated	[2]	[2]
2	Not indicated	Not indicated	[1]	[1]
4.25	129SvEv:Black Swiss	Not indicated	[20]	[3]
3.5	129SvEv:Black Swiss	Not indicated	[21]	[3]
6.4	129SvEv:C57BL/6J	Not indicated	[4]	[2]
10[a]	129SvEv:C57BL/6J	SPF	[7]	[7]
5	129SvEv, 129SvJ:C57BL/65	Non-SPF	[9]	[2]
4	129SvEv:C57BL/6J	Non-SPF	[10]	[2]
2.5	129SvEv	Not indicated	[8]	[2]
7	129SvEv:C57BL/6J	Not indicated	[5]	[2]
4	129SvEv	Not indicated	[22]	[2]
7.5	129SvEv	Not indicated	[6]	[2]
12.5	C57BL/6J	SPF	Unpublished data	[2]

[a] *Atm* deficient mice carry Atm^{y} mutation. Although no *ATM* protein was detected by using antibodies against C-terminus and N-terminus of the *ATM* protein, the authors do not exclude that low levels of aberrant *ATM* protein can be produced from one of the alternatively spliced transcripts detected in $Atm^{y/y}$ mouse tissues by RT-PCR. $Atm^{y/y}$ mice share the phenotype reported for other *Atm* mutant lines including growth-retardation, infertility, immunodeficiency and development of thymic lymphomas.

irrespective of type of facility, which is sometimes observed when a research lab moves to another research center. As an example, $Rad50^{S/S}$ mice held in SPF environment at the University of Wisconsin Medical School showed 6.62% incidence in lymphoma, however only 1.2% mice from the same colony developed lymphoma also in an SPF facility at the Memorial Sloan Kettering Cancer Center in New York ([13] and personal communication with John Petrini).

2. Differences in rodent diets

As another factor possibly contributing to variations in tumor onset and longevity of experimental animals, may be differences in rodent diets. It has been clearly established that caloric restriction reduces the cancer rate and prolongs survival of laboratory animals [14–16] possibly through reduced oxidative stress [17]. Rodent diets vary in their metabolizable energy content, and the extent of this variation depends on the macronutrient make-up. For example, the total fat range in standard diets falls between 3.5 and 12% (comparison of rodent diets manufactured by Harlan and Teklad, LabDiets, TestDiets, ProLab). Not only the content but also the type of fat differs in animal food. In some diets, all fat is derived from soy oil, while in others, animal fat is added, hence the difference in saturated versus nonsaturated fatty acids and absence of presence of cholesterol. There is evidence that diets enriched in vitamins and minerals can extend the longevity of mice [18,19]. The concentration of antioxidant vitamin E in standard diets varies from 35 to 134 IU/kg. In addition, diet ingredients are often chemically different and therefore, processed by the body differently. Purified diets contain refined ingredients like casein, sucrose, cornstarch, cellulose, and so on, while grain-based diets contains complex ingredients like corn, wheat, soybean meal. Ingredient differences, refined versus complex, can affect digestion, absorption, availability, hormonal release, and associated metabolism (personal communication with nutritionists from Harlan and Tecklad). No information on the diet was given in any of the *Atm* references discussed above and shown in Table 1. An increasing understanding that nutrients modulate disease and longevity and the fact that a variety of standard diets is available suggest that diet information should be included in research publications about phenotypes of transgenic rodents.

In summary, phenotypic differences of the same transgenic mice are sometimes observed in different laboratories and it is difficult if not impossible to determine causes thereof. In an attempt to get some insight into this matter, we suggest that authors in their articles supply information on the type of animal facility and diet used. Any other additional information that authors find relevant may turn out to be helpful too.

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