Grouped caging predisposes male mice to ankylosing enthesopathy

S Weinreich, J Capkova, B Hoebe-Hewryk, C Boog, P Ivanyi

Abstract
Objective—To evaluate the number of males per cage as a possible risk factor for murine ankylosing enthesopathy (ANKENT)—a spontaneous joint disease with parallels to human seronegative spondylarthropathies—since ANKENT shows incomplete penetrance of genetic susceptibility factors among individuals living in a stable environment.

Methods—Frequency of ANKENT was compared among males housed with females, with other males, or alone.

Results—In three independent cohorts, a trend was observed that males housed with females rarely develop the disease, in contrast to males housed with other males (P < 0.25, P < 0.05, and P < 0.01). Furthermore, no males caged alone developed ANKENT, whereas disease did occur in males grouped together (P < 0.01). When healthy males (retired breeders) were recaged either alone or with other males, ANKENT developed among the grouped males only (P < 0.005).

Conclusions—Caging males together is a relative risk factor for ANKENT. Grouped caging may perturb the immune system through endocrine pathways or modify microbiological load through behaviour (for example, infection due to biting).

Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology of the University of Amsterdam, Amsterdam, The Netherlands
S Weinreich
B Hoebe-Hewryk
C Boog
P Ivanyi

Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic
J Capkova

Correspondence to:
S Weinreich, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Pleinlaan 125, 1066 CX Amsterdam, The Netherlands.

Accepted for publication 15 April 1996


Murine ankylosing enthesopathy (ANKENT) is a spontaneous joint disease with numerous parallels to the human seronegative spondylarthropathies. These include age distribution, male predominance, characteristic pathology, and the presence of genetic risk factors in the major histocompatibility complex (MHC): notably, transgenic HLA B27 is a relative risk factor for ANKENT.1 However, the aetiology of ANKENT and the human seronegative spondylarthropathies, as well as the role of HLA B27, remains unclear. Despite intensive research, it is still speculative whether microbial infection is the underlying trigger of these diseases, and the definition of other non-genetic risk factors in human disease has been elusive. The animal model of ANKENT shows partial penetrance of genetic susceptibility factors, that is, individual variation in disease susceptibility occurs among genetically identical individuals living in a stable environment. This situation has facilitated the analysis of several non-genetic variables as possible risk factors. For example, increasing maternal age has been shown to be a protective factor in ANKENT.3

The basis for this phenomenon, and its relevance to human disease, requires further study.

In the course of studying several different groups of transgenic mice for the occurrence of ANKENT, a trend was observed that male breeders, that is, males caged with females but not with other males, rarely develop the disease, in contrast to males housed with other males. This paper describes experiments to test whether the number of males per cage affects the risk for disease.

Methods

The C57BI/10 (B10, H-2b) and B10.BR (H-2b) strains containing the transgenes HLA B2702 and human β2 microglobulin (hu-β2-m) have been described previously.1 In addition, an HLA B2702 + hu-β2-m transgenic, mouse β2 microglobulin knockout (muβ2m-ko) strain was studied.1 All transgenic mice reported in this study contained both the B2702 and hu-β2-m transgenes.

BREEDING AND HOUSING

Experiments I and II were performed in Amsterdam. All these mice were bred and housed in a conventional facility, except the non-transgenic B10.BR mice, which were purchased from OLAC (UK) at the age of four to six weeks. All mice were housed in the same room and received the same care.

Experiment III (retired breeders) was performed in Prague. Mice were bred and housed under SPF conditions while they were used as breeders; when they were recaged for the experiment, they were moved to a conventional facility.

SCORING FOR ANKENT

This was performed as described previously,1 once a month in experiments I and II and once a week in experiment III. Only males were used in this study, since ANKENT occurs predominantly among males.1

STATISTICAL METHODS

Statistical analysis was performed using the χ2 method or Poisson distribution,4 or by Lee Desu analysis.5

Results

The general rule for housing male mice is that they can be caged together, provided the group is formed before sexual maturity, and that no females are present. After sexual maturity, males from different cages are not usually put together, nor are multiple mature males housed together, due to aggression. Thus three
Table 1 Caging schemes used for males

<table>
<thead>
<tr>
<th>Designation of male(s)</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solitary</td>
<td>d</td>
</tr>
<tr>
<td>Breeder</td>
<td>d+*γ(γγγγ)</td>
</tr>
<tr>
<td>Grouped</td>
<td>d d d d</td>
</tr>
</tbody>
</table>

1 A male can be placed with one or more females. 
2 Males placed together before sexual maturity.

Table 2 AKNENT frequency in relation to caging scheme

<table>
<thead>
<tr>
<th>Strain</th>
<th>B27 + H-2 M</th>
<th>Solitary Breeder</th>
<th>Grouped</th>
</tr>
</thead>
<tbody>
<tr>
<td>B10</td>
<td>b +</td>
<td>0/16 (20/89)</td>
<td>22</td>
</tr>
<tr>
<td>ko B10</td>
<td>b +</td>
<td>1/11 (15/54)</td>
<td>28</td>
</tr>
<tr>
<td>B10.BR</td>
<td>k +</td>
<td>0/9 (85/166)</td>
<td>46</td>
</tr>
<tr>
<td>B10.BR</td>
<td>k -</td>
<td>0/34 (7/36)</td>
<td>19</td>
</tr>
<tr>
<td>B10</td>
<td>b -</td>
<td>0/22 (9/29)</td>
<td>31</td>
</tr>
</tbody>
</table>

1 Affected out of total (%). 
2 Retired breeders

P<0.05; P<0.01; P<0.005

Discussion

The data presented in this paper show that males caged together have a significantly higher risk for AKNENT than males caged alone or as breeders (that is, a single male but in the presence of females). The comparison of grouped males and initially healthy breeders has the disadvantage that these two groups are not absolutely matched for age, suggesting that they might have a differential risk for disease. However, previous studies have shown that disease risk is stable up to the age of nine months, so that this factor can be excluded.

In solitary B10.BR males, no AKNENT cases at all occurred, indicating that they may enjoy absolute protection from the disease. Among breeders, that is, males caged with females for a certain period, one out of 36 mice developed AKNENT, and one out of six mice who had started breeding with unilateral AKNENT subsequently developed AKNENT on the other side. Thus solitary caging may abrogate the risk for AKNENT altogether, whereas caging with females reduces disease risk; alternatively, caging with females may also abrogate the risk altogether, presuming that the single diseased breeder was already developing AKNENT when
Ankylosing enthesopathy with grouped caging

he was selected and moved to a cage with females. An important difference between the solitary males and the breeders is the age at which they were separated from their original male cage mates. For the solitary males this occurred at age four to six weeks, whereas for breeders it occurred later (six weeks and up). The data suggest that ANKENT is triggered by events or conditions operating after the age of four to six weeks, and that these events or conditions continue to present an active risk up to a much higher age. This notion is supported by the fact that 31% of retired, healthy breeders who were recaged with other males developed ANKENT, in contrast to retired healthy breeders who were recaged alone, and did not develop the disease.

It has previously been reported that there is sometimes an interval of several months between the occurrence of ANKENT on the first and the second hind paw. In the present study it was found that progression from unilateral to bilateral disease occurred in one breeder, six months after the breeding period began.

HORMONAL DIFFERENCES BETWEEN SOLITARY AND GROUPED MALES

Sex hormones are likely to play a role in susceptibility to ANKENT: the disease is unequally distributed between males and females, and it begins only after sexual maturity. However, we found no significant difference in testosterone levels between solitary and grouped males, nor between healthy and affected males (not shown). Experiments are in progress to examine whether castration reduces the risk for ANKENT.

It is also possible that grouped caging may affect other endocrine factors which indirectly increase the risk for ANKENT. In many species, the neuroendocrine system is known to interact with the immune system through adrenal and other hormones. A preliminary study revealed no difference in adrenal gland weights between solitary and grouped males, nor between healthy and ANKENT affected males (not shown). The effect of adrenalectomy on ANKENT susceptibility requires further study.

Interestingly, hormonal and behavioural factors have been shown to play a role in susceptibility to another naturally occurring murine joint disease, namely DBA/1 associated arthritis. DBA/1 males are protected from spontaneous arthritis by castration; this effect can be reversed by injection of testosterone. Moreover, the number of males per cage clearly affects disease development. As with the ANKENT model, male breeders do not develop the disease and, when they are recaged together at a late age, they rapidly develop arthritis. The disease in DBA/1 males is an osteoarthritis which begins in the toes and sometimes progresses to the ankles, whereas ANKENT is an enthesopathy which is exclusively localised in the ankle and tarsal area. Although DBA/1 arthritis and ANKENT have different pathological and genetic characteristics, some common hormonal or behavioural factors appear to influence susceptibility.

THE ROLE OF MICRO-ORGANISMS IN ANKENT

Since ANKENT did not occur among solitary mice, it might be suggested that the disease is a result of infection, since animals caged together are more likely to spread the disease to each other. Despite extensive efforts, previous research has provided no indications that mice with ANKENT carry specific pathogens or different commensal flora from healthy mice, nor has ANKENT ever been induced by bacteria. In the course of the present study, intestinal flora of solitary and grouped males was compared, but no difference was found between the two groups (not shown). There was also no indication among grouped males that ANKENT cases were clustered in particular cages.

Nevertheless, it cannot be formally excluded that ANKENT is triggered by infection. Studies with germ-free mice will resolve this issue.

So far, the risk induced by caging males together is not understood. These conditions could perturb the immune system through endocrine pathways or modify microbiological load through behaviour (for example, infection due to biting). An improved definition of hormonal and behavioural aspects of ANKENT susceptibility, as well as a study of disease susceptibility in germ-free animals, may provide a better understanding of the aetiology of this disease.

The authors gratefully acknowledge T. Jansen Hendriks for animal care, Dr P. Toivanen and Dr E. Eerola for gas chromatographic analysis of stools, Dr F. Shuter and J. Keyzer for measuring testosterone levels, W. Schaalberg for Lee-Desus analysis, and Dr H. Huitinga for helpful discussion. This work was financially supported by the Dutch Rheumatism Foundation, grant 92/CR/385 and the Commission of the European Communities, grant No ERBCIIPDTCT 940290.


Grouped caging predisposes male mice to ankylosing enthesopathy.

S Weinreich, J Capkova, B Hoebe-Hewryk, C Boog and P Ivanyi

doi: 10.1136/ard.55.9.645

Updated information and services can be found at:
http://ard.bmj.com/content/55/9/645

**Email alerting service**

*These include:*
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/