Maternal age influences risk for HLA-B27 associated ankylosing enthesopathy in transgenic mice

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Abstract
Objectives—To study further the temporal clustering of ankylosing enthesopathy (AE) noted originally during a study of the influence of mouse major histocompatibility complex (MHC) H-2 and transgenic HLA-B27 on the frequency of AE.

Methods—The relationship between maternal age at littering and frequency of AE was analysed.

Results—Mice born to mothers aged eight months or older had a significantly lower disease frequency of AE than mice born to mothers younger than eight months of age. This phenomenon was observed in three independent cohorts evaluated to date (p < 0.01, 0.025, and 0.05).

Conclusion—Maternal age is a novel, non-genetic risk factor as defined in relation to an MHC associated enthesopathy. Its mode of action and relevance to human disease require further investigation.

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The cellular and molecular basis of disease association with HLA-B27 remains unresolved. One approach to the study of this problem has investigated mice or rats with transgenes for human B27 and human β₂-microglobulin (huβ₂-m). Recently, a new mouse model for human spondyloarthropathies was described in HLA-B27 transgenics, namely mouse ankylosing enthesopathy (AE). Briefly, AE is a spontaneous joint disease of unknown aetiology, affecting primarily males between the ages of three and nine months. A mild inflammation of the hind paws progresses rapidly to irreversible stiffening as a result of bony outgrowth at the entheses of the ankle and tarsal joints. Genetic predisposition to AE is influenced by strain background (non-major histocompatibility complex (MHC) genetic factors) and H-2 haplotype (H-2 is the mouse equivalent of the HLA system in humans). Furthermore, a transgene for HLA-B27 is an additional relative risk factor for AE.

In addition to genetic factors which confer a relative risk for AE, environmental or other biological factors must dictate why only certain individuals develop the disease. Despite extensive analysis, there is no evidence to date that acute infection triggers AE. Thus other parameters might operate as risk factors or triggers among genetically susceptible, inbred strains, housed under uniform conditions. It has previously been observed that when a pool of breeding pairs is used to produce multiple litters for an experimental cohort, AE sometimes occurs in temporal clusters. For example, in a group of 76 males born consecutively from a single pool of breeding pairs, 64% of the first 17 animals developed AE, whereas in the following 59 animals only 12% developed the disease. With this type of breeding scheme, the only variable which is known to change in time is the increasing age of the breeders. We therefore analysed more thoroughly the kinetics of the occurrence of AE in relation to the age of the mother, studying the effect of maternal age in three cohorts of mice which were originally bred for genetic studies on the role of the B27 transgene and huβ₂-m as relative risk factors for AE.

Methods
For each cohort, a large majority of females produced multiple litters in the course of time. The genetic aspects of breeding schemes A, B, and C were explained in detail elsewhere. Briefly, each cohort was bred to contain littermates segregating for the B27 and huβ₂-m transgenes. Only male progeny were observed for AE. Animals were screened at least once a month as described previously, to the age of 10–12 months.

COHORT A (F₁)
F₁ hybrids of two H-2 congenic C57Bl10ScSn strains, namely B10.BR (H-2ª) and B10 (H-2ª) doubly transgenic for B27 and huβ₂-m were bred. In cohort A, the mothers in the parental generation were individually numbered and were approximately 2-5 months old when they started breeding.

COHORT B (F₂)
(B10.BR × B27 and huβ₂-m transgenic B10)F₂ mice were bred. Breeding was carried out with individually numbered F₁ parents for which the birth records had been maintained.

COHORT C (β₂-M KNOCKOUTS)
The establishment of β₂-m knockout mice has been described previously. Briefly, transgenes for B27 and huβ₂-m were introduced into β₂-m knockout mice by appropriate breeding crosses (manuscript in preparation). Parents of
Maternal age and HLA-B27 associated ankylosing enthesopathy in mice

the experimental cohort were individually numbered, and their birth records were maintained.

STATISTICAL ANALYSIS
Statistical significance was tested by the χ² method.4

Results
For the analysis of the effect of the mother’s age on the frequency of AE, cohorts A, B, and C are evaluated separately. Within each cohort, all progeny were considered together. This was justified because there was no indication that maternal age affected the Mendelian segregation of genotypes (data not shown).

The frequency of AE in each of the three cohorts was: cohort A (F₁) (n = 183) 59%; cohort B (F₁) (n = 355) 23%; cohort C (β₅₇m knockout) (n = 238) 17%. (The differences among cohorts were attributable to differences in H-2, transgenic B27, and the β₅₇m knockout phenotype, but this paper will not address these issues.)

The figure shows the kinetics of the frequency of AE in relation to maternal age. With slight fluctuations, the frequency of AE declined progressively from the maternal age of six months onwards, reaching zero by age 10 months. In order to draw a statistically based conclusion, the frequency of AE in each cohort was compared using the maternal age of eight months as reference point (figure). The table shows that the frequency of AE among mice born to mothers younger than eight months was significantly greater than that among mice born to mothers eight months of age or older (p < 0.01, 0.025, and 0.05 for cohorts A, B, and C, respectively). There was no indication that maternal age affected the age of disease onset or disease severity (data not shown).

Discussion
This analysis combined various genotypes within each of the cohorts A, B, and C. However, there was no indication that maternal age differentially affected disease risk for B27 positive or negative animals (data not shown). The results did demonstrate a diminished risk for AE in relation to increased maternal age. It may be suggested that, in conjunction with the increase in maternal age, microbiological or other environmental conditions in the room in which the experiments were performed also changed over time, producing an additional influencing factor. However, we consider this possibility to be highly unlikely because the breeding of cohorts A, B, and C overlapped only partially, and was carried out over a period of three years.

A decrease in the frequency of AE with increased maternal age having been established, several questions necessitate further experimental work. To determine whether maternal age is a direct risk factor or a reflection of a birth order effect, the frequency of AE must be compared between progeny of young and aged primiparous females. An intriguing possibility is that an age related increase in maternal antibody levels results in increasing protection of offspring against a ubiquitous, potentially arthritogenic microorganism. It is tempting to link the observation that the maternal age effect reached its maximum at 10 months, with the earlier observation that disease penetrance among susceptible males was also virtually complete at age nine months. This might imply that, during the ages of about six to 10 months, mice of both

Frequency of ankylosing enthesopathy (AE) in cohorts A, B, and C, according to maternal age

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Genotype†</th>
<th>Maternal age (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>≤ 7</td>
</tr>
<tr>
<td>A</td>
<td>B10 BR × tg B10 F</td>
<td>98/155 (63%)</td>
</tr>
<tr>
<td>B</td>
<td>B10 BR × tg B10 F</td>
<td>73/280 (26%)</td>
</tr>
<tr>
<td>C</td>
<td>(mu2+-m knockout B10) × tg mu2+-m knockout B10)</td>
<td>30/135 (22%)</td>
</tr>
</tbody>
</table>

Values are number affected/total (%).† Origin of males screened for (AE): tg = transgenic for B27 and mu2+-m. *p < 0.05; **p < 0.01.
genders gradually form protective antibodies, and that females transfer them to their young. Other studies in mice have demonstrated that female idiotypic antibody networks affect the immune response of offspring well into adulthood. In humans, age related changes in antibody levels in postpubertal women have been demonstrated in conjunction with the observation that young maternal age may be a relative risk factor for group B streptococcal disease.

Except for the strong association for age, gender, and HLA-B27, no other biological or environmental factors have been defined in relation to the occurrence of human spondyloarthropathies. With respect to rheumatoid arthritis (RA), it was recently reported that in a group of 115 patients, the majority of those with RA belonged to the early birth ranks. Maternal age and birth order also require systematic study in patients with spondyloarthropathies.

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