Gender-related differences in susceptibility, early virus dissemination and immunosuppression in mice infected with Friend murine leukaemia virus variant FIS-2

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An emerging amount of data indicates a correlation between gender-related factors and regulation of virus infection and supports what is known in clinical circles, that these topics are of great importance in many infectious diseases. In the present study we found that young adult NMRI male mice are more susceptible to infection by a variant of Friend murine leukaemia virus, FIS-2, than are female mice. We observed that the level of virus in serum, bone marrow and spleen was initially higher in male mice. Male mice were also more susceptible to FIS-2-induced immunosuppression. These results indicate a more efficient virus replication and dissemination in male mice. Studies with recombinant viruses between FIS-2 and the prototype Friend murine leukaemia virus revealed that FIS-2 LTR is one major factor contributing to the observed gender differences. A possible sex hormone influence on FIS-2 transcription due to the presence of a glucocorticoid response element in FIS-2 LTR is discussed.

Several studies suggest that progression in retrovirus diseases may largely depend on events occurring shortly after infection (Ho et al., 1996; Lifson et al., 1997; Mellors et al., 1996; Sarzotti et al., 1996; Watson et al., 1997). Antigen level, virus genetic factors and host factors that exert their effects prior to full development of specific immune responses can all be critical determinants for the immediate rate of virus dissemination and establishment of infection. Besides interindividual host differences, gender-related differences in susceptibility to virus infection and disease outcome have been reported, but not intensively studied (Gillespie & Roskow, 1968; for review, see Whitacre et al., 1999, and references therein; simian immunodeficiency virus-related: Marx et al., 1996; Smith et al., 2000; Sodora et al., 1998; human immunodeficiency virus-related: Farzadegan et al., 1998; Sterling et al., 1999). This is of special relevance to retrovirus infections, in which transmission most often occurs sexually or from mother to child. The Friend murine leukaemia virus (F-MuLV) model has proven valuable for studying general virological and immunological aspects of early phase in mammalian retrovirus infection (for review see, Chesbro et al., 1990; Hasenkug & Chesbro, 1997; Hasenkug & Dittmert, 2000; Soldaini et al., 1989). In the present study we therefore used a variant of this model to study the effect of host gender on susceptibility to murine retrovirus infection.

The viruses used in this study included a molecular clone of an immunosuppressive and low leukaemogenic variant of F-MuLV, FIS-2 (Dai et al., 1994; Faxvaag et al., 1993), the prototype F-MuLV, clone 57 (Troxler et al., 1980), and two chimeras, RE3 and RE4. Chimera RE3 contains FIS-2 LTR in an F-MuLV clone 57 background and chimera RE4 contains F-MuLV clone 57 LTR in an FIS-2 background (Dai et al., 1998). In all experiments we used 6- to 10-week-old male and female NMRI mice (an outbred strain where all mice have the same genotype of MHC, H-2k) purchased from Bomholt Gaard Breeding Research Center, Rye, Denmark, and kept in groups (<10) under conditions of controlled temperature and 12:12 h light/dark cycle, with food pellets and water ad libitum.

In the first experiment we inoculated male and female mice intraperitoneally with various doses of FIS-2. At 6, 8, 10 and 14 days post-infection (p.i.) groups of mice were bled from the cervical vein under anaesthesia (1:1 mixture of intraperitoneally or subcutaneously administrated hypnorm: dormicum) and then sacrificed by cervical dislocation. Virus in serum was detected by co-culturing serum with subconfluent monolayers of NIH 3T3 cells cultured in 24-well plates. The cultures were passed (trypsinized and diluted 1:10) three times before positive cells were detected by indirect membrane immunofluorescence using a mix of monoclonal antibody against gag-encoded MA protein (antibody 34) and mono-
Fig. 1. Incidence of viraemia and detectable productive infection of splenocytes in young adult female mice (left panels) and young adult male mice (right panels) at 6, 8, 10 and 14 days p.i. with different doses of FIS-2. The titre of the undiluted stocks was $3.2 \times 10^6$ IU/ml. The bars in the upper panels represent percentage of mice with detectable viraemia per dose point (6 days p.i. and 8 days p.i., $n = 2$–5 per group; 10 days p.i. and 14 days p.i., $n = 5$–13 per group; total female mice, 93; total male mice, 102). The bars in the lower left panel show percentage of female mice with detectable infection in the spleen at 6, 8, 10 and 14 days p.i. with 640, 6400 or 64000 IU of FIS-2 (6 days p.i. and 8 days p.i., $n = 2$–4 per group; 10 days p.i. and 14 days p.i., $n = 7$–9 per group; total of 60 female mice). The right lower panel shows incidence of detectable infection in male mice (total of 79 male mice; 6 and 8 days p.i., 4 mice per group; 10 and 14 days p.i., 6–13 mice per group).

clonal antibody reactive with F-MuLV gp70 (antibody 48) (Chesebro et al., 1981). The assay detection sensitivity was 50 infectious units (IU)/ml serum (20 µl serum per well) and has been described previously (Dai et al., 1998).

We observed that a much lower infective dose was required to obtain the same incidence of early viraemia in male compared to female mice (Fig. 1, upper panels). Viraemia in male mice was initially detected at 6 days p.i. with 640 IU. A similar incidence of detectable viraemia in female mice emerged first between days 8 and 10 p.i. with 6400 IU. Since infection in the spleen indicates a successful establishment of primary infection, we prepared single cell suspensions of splenocytes and co-cultivated them with NIH 3T3 cells ($2 \times 10^5$ splenocytes per well) to test for productive infection by indirect immunofluorescence detection. The gender difference in initial viraemia was mirrored in the fractions of mice with detectable infection in the spleen (Fig. 1, lower panels).

Looking at the fractions of mice with detectable viraemia and infection in the spleen, the gender difference was most profound at 6 and 8 days p.i. and faded at 10 and 14 days p.i. We therefore wanted to investigate whether there was a gender difference in virus level after established infection. Seven-week-old male and female mice were inoculated with 36000 IU of FIS-2. Groups of mice were sacrificed at 8, 11 and 14 days p.i. Viraemia titres were calculated by end-point dilution as described by Grist et al. (1990) (Fig. 2A, first panel). Virus expression in bone marrow and in the spleens was determined by immunohistochemistry (Fig. 2A, B). Eight days p.i. there was a significant difference in viraemia titres between male and female mice ($P = 0.02$). The level of virus expression in bone marrow and spleen red and spleen white pulpa was low in both male and female mice tested. At 11 days p.i. there was no significant difference in viraemia titres ($P = 0.230$), but now the level of virus expression in bone marrow and spleen was highest in male mice. We observed a gradual rise in virus expression in both bone marrow and spleen red pulp from day 8 to day 14 p.i. Positive spleen white pulpas were only detected in spleens with high levels of virus expression (Fig. 2A, B, bottom panels).

Since it was previously shown that rapid suppression of primary antibody response against a T-cell-dependent antigen, SRBC, is associated with FIS-2 infection (Dai et al., 1994, 1998; Faxvaag et al., 1993) we further investigated whether the observed gender-related virus-dose sensitivity could directly affect the susceptibility to FIS-2-induced immunosuppression. The assay, in which the number of B-cells producing anti-SRBC
Gender differences in FIS-2 infection

Fig. 2. (A) Level of detectable virus expression in serum, bone marrow and spleen at 8, 11 and 14 days p.i. with FIS-2. Seven-week-old female (○) and male (●) mice were inoculated intraperitoneally with 36,000 IU of FIS-2. The titre of the undiluted stock in this experiment was $1.8 \times 10^7$ IU/ml. Immunohistochemistry was performed by immunofluorescence staining.
is determined by using a slide monolayer technique, as has recently been described in detail (Dai et al., 1998). In the present study, we inoculated young adult male and female mice with various dilutions of FIS-2 stocks. Both infected and uninfected control mice were immunized with SRBC 10 days p.i. and the primary antibody response against SRBC was assayed 4 days later. Mice with relative plaque-forming cell (PFC) values below 10% of the average PFC value in the corresponding control group were considered immunosuppressed. As shown in Table 1, low virus inoculates caused a failure in the primary antibody response against SRBC more efficiently in male mice than in female mice. None of the male mice inoculated with 6400 and 640 IU could generate B-cells producing primary antibodies to SRBC. In comparison, a dose of 10000 IU was necessary to achieve the same extent of immunosuppression in female mice. Both actual PFC and PFC expressed as a percentage of the corresponding control group were compared between groups of male versus female mice given 6400 IU \( (P = 0.039) \) and 640 IU \( (P = 0.00) \) and gave identical significance levels. When we directly compared actual PFC in the different control groups no significant difference was found \( (P = 0.245–0.867) \). Hence, the significant gender difference in PFC was not due to different immune responses in male and female mice.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Virus dose</th>
<th>Virus-inoculated mice</th>
<th>Corresponding control mice</th>
<th>( P^\dagger )</th>
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<tbody>
<tr>
<td>Male</td>
<td>6400</td>
<td>1·3 [0·9] [7/7]</td>
<td>95·0 [19·9] [0/4]</td>
<td>0·003</td>
</tr>
<tr>
<td></td>
<td>6400*</td>
<td>0·0 [0·0] [6/6]</td>
<td>75·0 [7·8] [0/9]*</td>
<td>0·000</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>41·4 [15·0] [1/5]</td>
<td>104·3 [19·4] [0/8]</td>
<td>0·630</td>
</tr>
<tr>
<td>Female</td>
<td>6400</td>
<td>0·25 [1·6] [8/8]</td>
<td>114·6 [36·6] [1/7]</td>
<td>0·133</td>
</tr>
<tr>
<td></td>
<td>640</td>
<td>59·7 [43·0] [5/7]</td>
<td></td>
<td>0·072</td>
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* Experiment II with male mice inoculated with 6400 IU.

\( \dagger \) Comparison of actual PFC values between virus-inoculated mice and corresponding control group.

Significant values are given in bold type.

Table 1. Primary antibody response against SRBC in male and female mice 2 weeks p.i. with different doses of FIS-2: mean PFC values and statistical analysis for groups of infected mice and controls

Since both normal plots and the Shapiro–Wilks W test for normality revealed that not all the groups are compatible with a normal distribution, the exact two-tailed Mann–Whitney U rank sum test was used as a non-parametric alternative to the \( t \)-test for comparing data from two independent groups. \( P \) values \( \leq 0.05 \) were considered significant. SPSS version 8.0 software was utilized for all statistical analysis.
Gender differences in FIS-2 infection

(A) Genomic structure of the non-permuted forms of the parental and chimeric viruses. Solid and open regions are derived from FIS-2 and F-MuLV clone 57, respectively. The positions of LTR, gag, pol and env are indicated. The arrowheads illustrate the number of direct repeats in the LTR region. (B) Viraemia titres in young adult male and female mice inoculated intraperitoneally with parental or chimeric viruses. The titres of the undiluted stocks were $1 \times 10^7$ (FIS-2 and RE4), $1 \times 10^8$ (RE3) and $5 \times 10^6$ (F-MuLV clone 57) IU/ml respectively and the doses of virus used are shown on the top of the dot plots together with the time of sacrificing and the total number of mice in each group. Blood was collected for analysis of viraemia titres and the spleens were removed and analysed as a further marker for virus infection. Each circle or square represents a mouse (total of 156) and solid points indicate mice with detectable infection in the spleen. Dashed lines indicate the detection of virus in the spleen.
by end-point dilution. As shown in the first panel of Fig. 3(B), there was a significant difference in viraemia titres between groups of male and female mice inoculated with FIS-2 (P = 0.004). In the groups of mice inoculated with an equal dose of F-MuLV clone 57 (Fig. 3B, last panel) there was a noticeable but not significant gender difference in viraemia titres (P = 0.079). Hence, the gender difference was more profound in FIS-2 infection than in F-MuLV infection.

Since retroviral LTR is the major determinant that controls replication through transcription, we used recombinant viruses between FIS-2 and F-MuLV clone 57 to investigate whether FIS-2 LTR contributed to the marked gender differences in FIS-2-inoculated mice. When FIS-2 LTR was replaced with F-MuLV LTR as in the RE4 chimeric virus (Fig. 3A, B, third panel), we could not detect any significant gender difference either at high virus dose (P = 0.690) or at low virus dose (P = 0.429). Additional indication for FIS-2 LTR involvement in the gender differences was given in experiments with the RE3 chimeric virus. When F-MuLV LTR was replaced with FIS-2 LTR (Fig. 3A, B, second panel), we could detect a marginally significant gender difference at a high virus dose (P = 0.05). None of the female mice and 3 out of 10 male mice inoculated with low virus dose had detectable infection at 6 days p.i. At 10 days p.i. with 2000 IU of RE3, all male mice (n = 5) but only 3 out of 6 female mice had detectable infection. All mice inoculated with 20000 IU had detectable infection at 10 days p.i. (data not shown).

These results indicated that gender-related factors could have a more direct effect on the activity of FIS-2 LTR than on the activity of F-MuLV LTR. The nucleotide sequence of FIS-2 LTR shows high homology with that of F-MuLV LTR except for the deletion of one direct repeat and a few point mutations, including generation of a glucocorticoid response element, GRE (Dai et al., 1994). GRE mediates gene induction by glucocorticoids, progesterone and androgens, but not by oestrogens (Beato et al., 1989; Cato et al., 1986; Darbre et al., 1986; Otten et al., 1988; Schüle et al., 1988). It has been suggested that incorporation of a GRE within the LTR represents a common strategy among retroviruses that could serve to increase the transcription activity of their promoters (Beato et al., 1989; Miksicek et al., 1986). Several studies have reported regulation of gene activity by GRE in vitro by a broad spectrum of viruses including hepatitis B virus (Tur-Kaspa et al., 1986), human polyomavirus BK (Moens et al., 1994) and complex retroviruses such as bovine leukaemia virus (Niermann et al., 1997) and human immunodeficiency virus (Kolesničenko & Snart, 1992; Mitra et al., 1995; Soudeyns et al., 1993). Incorporation of a GRE in FIS-2 LTR might contribute to the significant gender difference in FIS-2 infection.

It is also possible that the number of target cells or the receptor level can be affected by the gender-related hormonal environment. It has been shown in rat quiescent liver cells that type C ecotropic retrovirus receptor can be upregulated by some hormones, including dexamethasone and insulin (Wu et al., 1994). Vassiliadou et al. (1999) showed in another study that progesterone-induced inhibition of chemokine receptor expression on peripheral blood mononuclear cells correlated with reduced infectiousness of human immunodeficiency virus type 1 in vitro. This study also suggested that progesterone could have negative effects on chemokine-mediated recruitment of lymphocytes and monocytes to mucosal epithelia. In the present study NMRImice were inoculated intraperitoneally with different virus clones, and one potential explanation for the observed general gender difference in susceptibility might be that sex hormones induce dimorphism in the recruitment of target cells at this site of infection.

In conclusion, we have shown that gender clearly is of importance in primary FIS-2 infection. The time from virus inoculation to detectable infection was shorter for male mice than for female mice and this gender-related sensitivity, probably due to high initial replication of FIS-2 in male mice, resulted in higher male susceptibility to FIS-2-induced immunosuppression. Studies with recombinant viruses between FIS-2 and the prototype F-MuLV, clone 57, revealed that the FIS-2 LTR was one major factor contributing to the observed gender difference, and we speculate that this might, at least in part, be due to the presence of a GRE in FIS-2 LTR. We now focus on identifying the cell types infected by FIS-2 during primary infection in male and female mice and investigate possible direct activation of FIS-2 LTR by sex hormones in these cells.

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References


Chesebro, B., Gehly, K., Cloyd, M., Brit, W., Portis, J., Collins, J. & Nishio, J. (1981). Characterization of mouse monoclonal antibodies sensitivity limit for viraemia, equivalent to 50 IU/ml serum. Employing the value log10 = 1.1 (detection limit) in replacement for the left-censored values, the viraemia titres were ranked and examined with the two-tailed Mann–Whitney U rank test for independent samples, using exact test option (corrects for ties) by SPSS version 8.0 software. P values are shown on the top of the dot plots and significant values (P ≤ 0.05) are given in bold type. The median in each group is indicated as a horizontal line. (*) The titres in two positive sera were not determined.
Gender differences in FIS-2 infection


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