An X-linked Locus Influences the Amount of Circulating Interferon Induced in the Mouse by Herpes Simplex Virus Type 1

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SUMMARY

Production of circulating interferon (IFN) was measured in inbred mouse strains following intravenous injection of herpes simplex virus type 1 (HSV-1). IFN titres reached maximal levels 2 to 3 h after injection of virus and a 10-fold difference was found between C57BL/6 mice and BALB/c, as high and low producers respectively. Mendelian analysis revealed that HSV-induced IFN production is governed by several loci, one of which is X-linked. The strain distribution pattern obtained from results in recombinant inbred lines and the results obtained in the congenic B6-C-H-28-If-1 strain furthermore indicated an absence of close linkage to If-1. It is concluded that the levels of HSV-induced early IFN production are influenced by several autosomal loci and one X-linked locus.

INTRODUCTION

Several genetic loci in the mouse influence the levels of circulating interferon (IFN) production in response to viruses. The loci controlling IFN production with one virus do not necessarily have an effect on interferon levels induced by another virus; for example, both Newcastle disease virus (NDV) and Sendai virus induce 10-fold higher levels of serum IFN in C57BL/6 than in BALB/c mice but segregation analysis and the use of recombinant inbred (RI) mouse strains have shown that different loci are involved in regulating IFN production in response to the two viruses (DeMaeyer & DeMaeyer-Guignard, 1979). Injection of herpes simplex virus (HSV-1) into mice also results in high levels of circulating IFN, and it could again be shown that the amount of IFN produced is influenced by the genotype of the host, in that C57BL/6 mice produce higher amounts of IFN than mice of other strains such as DBA/2 or BALB/c (Zawatzky et al., 1981). It was therefore of interest to determine the number of loci involved in the quantitative difference between high and low IFN responders to HSV, and the linkage of these loci to other If-loci. In this paper, we present evidence that early (between 2 and 3 h) IFN production in response to HSV is under the quantitative control of at least three loci, one of which is X-linked.

METHODS

Virus. Herpes simplex virus type 1, strain WAL (HSV-1 WAL), derived from a virus stock as previously described (Kirchner et al., 1978), was plaque-purified and propagated in RITA cells (a continuous line of monkey kidney origin screened for the presence of mycoplasma) in Medium 199 (Morgan et al., 1950), supplemented with 5% foetal bovine serum (FBS) at a multiplicity of infection (m.o.i.) of 0.02. Virus particles were recovered 24 h later from the supernatant of RITA cells by sedimentation at 27000 g for 1 h. Extraction and purification of infectious virus DNA and subsequent infection of BSC-1 cells (African green monkey kidney cells) were performed essentially as described by Graham et al. (1973), using the calcium phosphate precipitation technique. Plaques of HSV-1 could be identified 3 days after infection of BSC-1 cells with virus DNA. Virus was then propagated again in RITA cells at low m.o.i. (0.02) to obtain a new virus stock. Derived from the latter, the first passage at high m.o.i. (1.5) was used in all the experiments of this study. The titre of this preparation was 10^8 p.f.u./ml. It will be referred to below as HSV.

Interferon induction. Mice received an intravenous injection, into the retroorbital sinus, of 0.1 ml HSV...
suspension, corresponding to $10^7$ p.f.u./animal. Blood was drawn between 2 and 3 h later, from the same site, and after overnight storage at 4 °C, the serum was separated and stored at -20 °C. Before the interferon titrations, all sera were pre-diluted 1:10 in minimal essential medium (MEM) and acidified to pH 2 by the addition of 0.5 M-HCl. Four h of acid treatment was found sufficient to inactivate any HSV remaining in the serum. The decision to measure IFN 2 to 3 h after inoculation of the virus was based on the observation, from preliminary experiments, that optimal IFN induction was achieved at that time. Interferon titres reached maximal levels 2 to 3 h following virus inoculation, and no difference in kinetics of IFN production between parental strains (male C57BL/6 and BALB/c) could be observed. Titres remained at maximal levels for 6 h after virus infection and then declined slowly. No IFN production was observed in either C57BL/6 or BALB/c mice following intravenous injection of supernatants from uninfected RITA cells.

**Interferon assay.** All IFN titrations were performed using a microtitre assay in 96-well tissue culture plates (Falcon). Appropriate twofold dilutions of the acid-treated sera were incubated overnight in the presence of mouse L cells. The challenge virus, consisting of encephalomyocarditis virus (EMCV), was then added and the cell protection read 24 h later. Each serum was titrated in duplicate. One unit is defined as the reciprocal of the last serum dilution conferring at least 50% protection against destruction of the L cells by EMCV, and corresponds to 3 international reference units, based on comparable titrations of NIH mouse interferon preparation with reference number G-002-904-511. All units are expressed as log_{10} values.

**Mice.** The inbred strains used in this study were originally obtained from D. Bailey at the Jackson Laboratory, Bar Harbor, U.S.A., and have been maintained in Orsay, by brother–sister mating for several years. All mice were 2 to 3 months old at the time of the experiments.

**Statistical analysis of the data.** The log_{10} values of the titres were used for the analysis by Student's t-test (Dixon & Massey, 1969), since it has been shown previously that values for circulating IFN levels in mice have a close to normal distribution when plotted on a log_{10} scale (DeMaeyer & DeMaeyer-Guignard, 1979). This was confirmed in the present study, as can be seen in Fig. 1. In addition, the results were analysed non-parametrically by the Wilcoxon rank sum test (Hollander & Wolfe, 1973). In general, there was a good correlation between the outcomes of the two tests.

**RESULTS**

**Interferon titles in parental strains (BALB/c and C57BL/6)**

The mean titres in BALB/c and C57BL/6 mice differed by a factor of about 10. When males and females were analysed separately, the average titre of the females, both for BALB/c and C57BL/6, was slightly higher than the average titre of the males. For both strains, the difference between males and females was statistically significant ($P < 0.005$) (Table 1, Fig. 1).

**F1 generation**

When IFN titres of F1 progeny of reciprocal crosses were analysed, a striking difference between males and females was observed. For both crosses, BALB/c × C57BL/6, and C57BL/6 × BALB/c, the titres in the female progeny were significantly higher than the titres in the male progeny and much closer to the values of C57BL/6 than to those of BALB/c.

An analysis of the titres of the male progeny revealed a significant difference ($P < 0.01$) between the progeny of the two crosses in that the titres of the C57BL/6 × BALB/c progeny were higher than those of the BALB/c × C57BL/6 progeny. The female progeny of both crosses had comparable titres (Table 1, Fig. 1).

<table>
<thead>
<tr>
<th>Table 1. HSV-induced IFN titres in parental strains and F1 hybrids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Mean titre</td>
</tr>
<tr>
<td>Standard deviation</td>
</tr>
<tr>
<td>Number of mice</td>
</tr>
<tr>
<td>Level of significance</td>
</tr>
<tr>
<td>between ♀ and ♂</td>
</tr>
<tr>
<td>between F1 ♀ and ♂</td>
</tr>
<tr>
<td>between F1 ♂ and ♂</td>
</tr>
</tbody>
</table>

Between F1 ♂ and ♂ $P < 0.01$.
Interferon and herpes simplex virus

Fig. 1. Genetic analysis of HSV-induced circulating IFN levels. Titres were measured in parental strains, F1, F2 and backcross generations. The open symbols represent values obtained from female animals, the closed symbols males.

Table 2. HSV-induced IFN titres in F2 and backcross generations

<table>
<thead>
<tr>
<th>Strain</th>
<th>F2 (BALB/c × C57BL/6)</th>
<th>F1 (BALB/c × C57BL/6) × C57BL/6 backcross</th>
<th>F1 (BALB/c × C57BL/6) × BALB/c backcross</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \delta )</td>
<td>( \varphi )</td>
<td>( \delta )</td>
</tr>
<tr>
<td>Mean titre</td>
<td>3.17</td>
<td>3.29</td>
<td>2.58</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>2.68</td>
<td>2.82</td>
<td>0.36</td>
</tr>
<tr>
<td>Number of mice</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Level of significance between ( \varphi ) and ( \delta )</td>
<td>( P &lt; 0.001 )</td>
<td>( P &lt; 0.001 )</td>
<td>( P &lt; 0.001 )</td>
</tr>
</tbody>
</table>

**F2 generation: (BALB/c × C57BL/6) F1 × (BALB/c × C57BL/6) F1**

The IFN titres of the F2 progeny were also significantly different for males and females. Again, the titres were higher in the female progeny than in the male progeny, and the difference between the mean values of both was highly significant (\( P < 0.001 \)). Furthermore, the values obtained from males were spread over the whole range of BALB/c to C57BL/6 titres, whereas those from females were only of intermediate and of C57BL/6 type (Table 2, Fig. 1).
Fig. 2. Genetic analysis of HSV-induced circulating IFN levels. Titres were measured in recombinant inbred and congenic lines. The open symbols represent values obtained from female animals, the closed symbols males.
Interferon and herpes simplex virus

**Backcross generation**

(BALB/c x C57BL/6) F1 x C57BL/6

The female progeny of this backcross had IFN titres comparable to those of C57BL/6, whereas the titres of the male progeny were intermediate, between C57BL/6 and BALB/c values. Again, the difference between male and female values was highly significant (P < 0.001) (Table 2, Fig. 1).

(BALB/c x C57BL/6) F1 x BALB/c

About half of the titres of the female progeny fell within the range of the female BALB/c values whereas the rest were intermediate between female C57BL/6 and BALB/c. The same was true for male progeny when compared to male C57BL/6 and BALB/c mice (Table 2, Fig. 1).

**Recombinant inbred strains and the congenic B6-C-H-28-If-11 (HW94) strain**

The strain distribution pattern (SDP) of HSV-induced IFN levels was further examined in seven RI lines derived from C57BL/6 and BALB/c (Bailey, 1971).

For all these strains, the level of IFN production was intermediate between C57BL/6 and BALB/c. Again the females had higher IFN titres than the males, and the difference was statistically significant for six strains, the exception being the CXBD strain. The females of one RI strain (CXBE) produced values that came close to the female C57BL/6 values but were nevertheless significantly lower (P < 0.025). The values from males of another RI strain (CXBH) were close to the male BALB/c values, but still significantly different (P < 0.05) (Table 3, Fig. 2).

To look for possible linkage with If-1 (DeMaeyer & DeMaeyer-Guignard, 1969), HSV-induced IFN production was measured in the congenic B6-C-H-28-If-11 (HW94) strain (DeMaeyer et al., 1975). In this strain also the IFN production of females was significantly higher than that of males; however, the levels were not significantly different from C57BL/6 levels (Table 3, Fig. 2).

**DISCUSSION**

A striking feature of the analysis of HSV-induced IFN levels in the present study is the significantly higher titres obtained in female animals in all the crosses examined as well as in the parental, the RI and the congenic strains. There are several indications that this difference is X-linked. The most important evidence is the outcome of the determination of IFN levels in the male progeny of the F1 crosses in both directions. The fact that the IFN levels of the male progeny of the C57BL/6 x BALB/c cross are significantly higher than those of the progeny of the BALB/c x C57BL/6 cross clearly indicates an X-linked influence in this cross, since IFN levels are higher when the X-chromosome originates from C57BL/6 than from BALB/c mice.

This is further borne out by the IFN titres of female progeny of the F1 generation in both directions, which are on average 3.7 times (for C57BL/6 x BALB/c) and 6 times (for BALB/c x C57BL/6) higher than the titres of the male progeny, whereas the difference between average titre of males and females in the parental strains is only 1.5 times (BALB/c) and 2 times (C57BL/6). This indicates that, when two X-chromosomes are present, the influence of the high producer X-linked allele of C57BL/6 origin is dominant over all other alleles influencing HSV-induced IFN levels. This is also suggested by the outcome of the F2 and F1 x C57BL/6 backcross, in which the mean titre in the female progeny is about 3 times that of the male progeny. Analysis of the distribution of the titres of the male and female F2 progeny also reveals that several loci are involved, since, if we were only dealing with one locus, 25% of the female F2 progeny would be expected to have titres comparable to BALB/c values. This would correspond to 7 or 8 (i.e. 25%) animals, whereas in the observed result only 1 (i.e. 3%) of the progeny fell within the range of female BALB/c values. By the same reasoning, one would expect 75% of the male F2 progeny to have titres corresponding to male BALB/c or F1 values, which are indistinguishable because of the considerable overlap of the observed values; in fact, only 15 out of 30, or 50% fall within this range. The actual number of loci involved, in addition to the X-linked locus, cannot be accurately deduced from the segregation results, since too many
Table 3. **HSV-induced IFN titres in RI strains and in the congenic HW 94 strain**

<table>
<thead>
<tr>
<th>Strain</th>
<th>D</th>
<th>E</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
<th>K</th>
<th>HW94</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>2.43</td>
<td>3.09</td>
<td>2.63</td>
<td>2.53</td>
<td>2.62</td>
<td>2.62</td>
<td>3.00</td>
<td>3.33</td>
</tr>
<tr>
<td></td>
<td>0.44</td>
<td>0.24</td>
<td>0.31</td>
<td>0.17</td>
<td>0.20</td>
<td>0.35</td>
<td>0.34</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2.35</td>
<td>2.60</td>
<td>2.23</td>
<td>2.07</td>
<td>2.32</td>
<td>2.20</td>
<td>2.44</td>
<td>2.97</td>
</tr>
<tr>
<td></td>
<td>0.12</td>
<td>0.48</td>
<td>0.18</td>
<td>0.15</td>
<td>0.10</td>
<td>0.13</td>
<td>0.16</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>11</td>
<td>10</td>
<td>11</td>
<td>7</td>
<td>21</td>
</tr>
</tbody>
</table>

Level of significance between female and male:

- NS*  
- P < 0.005  
- P < 0.005  
- P < 0.005  
- P < 0.005  
- P < 0.005  
- P < 0.005

* NS, Not significant.
Table 4. Estimation of the number of loci involved in determining the levels of HSV-induced IFN production from segregation in the F2 progeny

<table>
<thead>
<tr>
<th>Number of loci</th>
<th>Female progeny: Expected percentage of BALB/c-type producers</th>
<th>Observed percentage</th>
<th>Male progeny: Expected percentage of BALB/c- and F1-type producers</th>
<th>Observed percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25%</td>
<td>3%</td>
<td>1</td>
<td>75%</td>
</tr>
<tr>
<td>2</td>
<td>6.2%</td>
<td>56%</td>
<td>2</td>
<td>56%</td>
</tr>
<tr>
<td>3</td>
<td>1.5%</td>
<td>42%</td>
<td>3</td>
<td>42%</td>
</tr>
</tbody>
</table>

assumptions concerning the relative dominance of the different alleles would have to be made; the segregation values suggest that two or three loci are involved (see Table 4). The results obtained in the RI strains confirm that the trait under study is under multifactorial influence, since none of the RI strains has a clear-cut parental phenotype, and for all strains the observed values are intermediate between BALB/c and C57BL/6. The SDP of HSV-induced IFN levels in the RI strains furthermore shows no obvious correspondence with any of the previously observed SDPs for IFN induction by NDV, mouse mammary tumour virus or Sendai virus, thereby suggesting lack of close linkage to any of the If-loci previously described. Lack of linkage to If-1 is furthermore confirmed by the results obtained in the B6-C-H-28~If-1 strain, in which the HSV-induced IFN titres were of C57BL/6 type rather than of BALB/c type. The conclusion of this study, therefore, is that the levels of early HSV-induced IFN production are influenced by several (possibly two) autosomal loci and one X-linked locus: none of these is closely linked to If-1, and probably not to any of the other If-loci either. The latter point, however, will have to be confirmed by further linkage studies, since, with the exception of If-1, no congenic strains for the other If-loci are available.

These results have several implications: loci influencing levels of IFN production in the mouse have been previously described for other IFN-inducing viruses, but no evidence for X-linkage of these genes was observed. However, in the case of these other viruses, early IFN production was not studied in detail, since only IFN levels at later times (8 to 12 h) were compared. Therefore, the possibility that with some of the other inducers the level of early IFN induction is also to some extent under X-linked control can not be excluded. This point certainly merits further investigation, since a more general influence of the X-chromosome on the levels of IFN production with several viruses would have implications for the physiopathology of virus diseases in males and females.

A Mendelian analysis performed by Lopez (1980), using C57BL/6 and BALB/c mice as parental strains, provided evidence that primary resistance of C57BL/6 mice to HSV-induced encephalitis is mediated by two independently segregating loci; no evidence for X-linkage was reported in the analysis by Lopez. However, two recent reports have provided evidence for an X-linked locus or loci influencing resistance of mice to HSV-2-induced hepatitis (Mogensen, 1977) and HSV-2 infection (D. Armerding, personal communication) and we believe that these observations are directly relevant to the results reported here.

Several reports in the literature provide evidence for the existence of X-linked genes in man influencing susceptibility to Epstein–Barr virus (EBV), which belongs to the herpesvirus group. Males affected with the X-linked lymphoproliferative syndrome are uncommonly susceptible to infectious mononucleosis, and this disease often has a fatal course in such patients (Purtito, 1980). Interestingly enough, these males frequently have deficient natural killer cell activity (Sullivan et al., 1980). In addition, nasopharyngeal carcinoma, in which EBV has been implicated as a causative agent, occurs mainly in genetically predisposed males (for review, see Purtito, 1981). In view of the highly conserved nature of the X-chromosome, we believe that the possibility that herpesvirus-induced IFN production is X-linked also in man merits full investigation.

REFERENCES


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