The Effect of Gold Sodium Thiomalate in Adult Swiss/A2G Mice Infected with Togaviruses and Flaviviruses

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(Accepted 22 June 1987)

SUMMARY

Treatment of adult mice with gold sodium thiomalate made the normally non-lethal Semliki Forest virus and Sindbis virus infections lethal and increased the virulence of Langat and West Nile viruses. These changes were associated with an enhanced virus invasion of the brain. Depression of the humoral antibody response was not observed.

Gold sodium thiomalate (GSTM) is used for the treatment of rheumatoid arthritis (American Rheumatism Association, 1973), in the form of the thiocomplex. It enters the lysosomal compartment and inhibits peritoneal macrophage lysosomal enzyme activity (Persellin & Ziff, 1966; Mehta & Webb, 1982; Finkelstein et al., 1982). GSTM converts the normally avirulent A7(74) strain of Semliki Forest (SF) virus infection to a lethal infection, characteristic of that seen with the virulent L10 strain of SF virus. (Allner et al., 1974; Bradish et al., 1975; Oaten et al., 1980; Pathak & Webb, 1983).

The present study extends these findings by investigating the effect of GSTM on virulence in adult mice infected with various togaviruses and flaviviruses. These include Sindbis (SIN) virus, a togavirus which normally produces an avirulent infection, Langat (TP21 strain) and West Nile (WN) viruses, both flaviviruses, which exhibit a low neurovirulence when inoculated intraperitoneally (i.p.) but are highly virulent when inoculated intracerebrally (i.c.).

In the present study mice of approximately 4 weeks of age were inoculated i.p. with 10 mg/mouse GSTM 3 h prior to i.c. or i.p. inoculation of virus, or with virus only. SF and SIN viruses were inoculated i.c. and i.p.; Langat and WN viruses were inoculated i.p. The following virus dilutions were used: SF virus of either strain and SIN virus containing 10^5 intracerebral 50% lethal dose (ICLD_50)/ml, Langat and WN viruses containing 10^3 ICLD_50/ml.

Six mice from each group were sampled for brain, blood and peritoneal macrophages on appropriate days post-inoculation and were stored at -70 °C for virus estimation. The serum was stored at -20 °C for the measurement of antibody response. The virus infectivity of a sample was determined by diluting a 10% (w/v) suspension of the tissue in serial 10-fold steps in 0.75% bovine serum albumin (BSA) phosphate-buffered saline (PBS), each dilution was inoculated into five mice. All specimens containing SF L10, Langat or WN virus were inoculated i.c., 0.03 ml into 3- to 4-week-old mice, those containing SF A7(74) or SIN virus were inoculated i.c., 0.02 ml into 2- to 4-day-old mice. The infectivity was expressed as ICLD_50/brain or ml of blood and peritoneal macrophages, calculated by the method of Reed & Muench (1938).

Peritoneal macrophages were collected from peritoneal exudate of each mouse by allowing them to adhere to a pre-weighted Petri dish for 1 h at 37 °C (Oaten et al., 1980). The cells were consistently 95% macrophages as characterized by their adhering properties. The non-adhering cells were removed by rinsing the monolayer with Hanks' balanced salts solution and the Petri dish was then reweighed. The cells were resuspended in a volume of PBS with 0.75% BSA calculated to make a 10% suspension.

An ELISA based on the method of Voller et al. (1976) was used to measure the immunoglobulin (Ig)M and IgG response in serum to SF, SIN, Langat and WN viruses on
Short communication

Fig. 1. Virus titres within the brain of control and GSTM-treated 4-week-old mice after i.c. and i.p. inoculation with SF virus. O, GSTM + virus i.c.; O, virus only i.c.; ▲, GSTM + virus i.p.; △, virus only i.p. Each point represents the data from four mice and the vertical bars represent 1 mean standard error. (a) strain A7(74) of SF virus and (b) strain L10 of SF virus.

Fig. 2. Virus titres within the brain of control and GSTM-treated 4-week-old mice after inoculation i.e. and i.p. with SIN virus. O, GSTM + virus i.c.; O, virus only i.c.; ▲, GSTM + virus i.p.; △, virus only i.p. Each point represents the data from four mice and the vertical bars represent 1 mean standard error.

Table 1. Mortality (%) and average day of death in GSTM-treated and untreated infected mice*

<table>
<thead>
<tr>
<th>Virus</th>
<th>Route of inoculation</th>
<th>Mortality (%)</th>
<th>Average day of death†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treated</td>
<td>Untreated</td>
</tr>
<tr>
<td>SFV A7(74)</td>
<td>i.c.</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>SFV L10</td>
<td>i.p.</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>SIN</td>
<td>i.c.</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>i.p.</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>Langat</td>
<td>i.p.</td>
<td>50</td>
<td>15</td>
</tr>
<tr>
<td>WN</td>
<td>i.p.</td>
<td>80</td>
<td>30</td>
</tr>
<tr>
<td>GSTM control</td>
<td>i.p.</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

* Thirty mice were inoculated in each group.
† Values in parentheses are standard errors of the mean.
‡ P was calculated by the Student's t-test.

various days after infection. Results were recorded as the highest dilution (log_{10}) giving a reading significantly above that of serum from an uninoculated mouse and expressed as the mean value from four mice, using the standard error of the mean. The antigens were prepared by purifying the different viruses using the method described by Faulkner & McGee-Russell (1968).

Representative data on the mortality of mice and the average day of death are shown in Table 1. The results indicated that the illness produced by SF virus (both i.c. and i.p.), SIN virus (i.c. but not i.p.) and Langat and WN virus infection was highly potentiated following administration of GSTM. The treated infected mice developed signs of illness such as fur ruffling, weight loss, a marked decrease in their activities, and paralysis before death. GSTM on its own at this dose produced 3% mortality.

The course of viraemia and the peritoneal macrophage virus titres were not influenced by GSTM in any of the virus infections but followed closely the common pattern obtained in the virus control group. There was however, a significant difference in brain virus titres as shown in Fig. 1, 2, 3 and 4 between the two groups in most of the virus infections except in the i.p. infections of SF virus L10 and SIN virus.

A maximum increase in brain virus titres in the treated group relative to the untreated control
group of the following virus infections was recorded. All the values had $P < 0.001$. For SF virus, a maximum increase of $10^{4.5}$ in the i.c. infected group and $10^{4.9}$ in the i.p. infected group was found on days 4 and 5 after infection respectively. For SIN virus, an increase of more than $10^3$ between days 3 and 6 was found and for Langat virus infection and WN virus infection increases of $10^5$ to $10^6$ and $10^5$ to $10^6$ respectively were recorded between days 7 and 9 post-infection (Fig. 1a, 2, 3, 4).

There was no detectable depression of either IgM or IgG synthesis by GSTM in SF virus infection. This confirmed previous work by Allner et al. (1974), Oaten et al. (1980) using the A7(74) strain in A2G mice and by Gates et al. (1984) using the M9 strain of SF virus in C57BL/6 mice. In addition the present work shows that GSTM has no effect on antibody synthesis in SIN, Langat and WN virus infections. These results therefore suggest that the manifestation of virulence in GSTM-treated SF, SIN, Langat and WN virus-infected mice is not associated with antibody synthesis.

Results in this study show that treatment with GSTM only alters the course of infection in the central nervous system and not in the extraneural tissue. The mechanism by which GSTM can increase the virulence of certain viruses is not fully understood. Allner et al. (1974) and Oaten et al. (1980) suggested that the inhibition of lysosomal enzyme activity by GSTM in peritoneal macrophages may alter the functions of these macrophages in such a way that a normally non-lethal infection of SF virus A7(74) is rendered lethal. The role of macrophages in the expression of virulence has been further examined by the use of agents such as silica and anti-macrophage serum. These agents do not affect the virulence of SF virus A7(74) and Langat virus (Mehta, 1986). These results together with the fact that some gold compounds such as gold (III) dimercaptosuccinic acid inhibit macrophage lysosomal enzymes but do not render SF virus A7(74) infection lethal, indicate that macrophages may not be important in the expression of virulence by the A7(74) strain of SF virus and Langat virus in GSTM-treated mice (Mehta, 1986).

Pathak et al. (1976) and Pathak & Webb (1978) showed that the replication of the virulent L10 strain of SF virus in adult mouse brain was associated with a proliferation of membranous vesicles with mature virus particles budding from these. Very few cytopathic vacuoles and no mature virus particles were seen in the brain of adult mice infected with the avirulent A7(74) strain. The avirulent strain thus differs from the virulent in that it does not induce the proliferation of the membranous structures necessary for its replication. At the electron microscopical level, Pathak & Webb (1983) investigated the effect of GSTM on the replication of the avirulent A7(74) virus and observed that mature virus particles were associated with cytopathic vesicles in adult mouse brain. Furthermore, examination of the brain of adult mice
given GSTM alone demonstrated an increased proliferation of smooth membranes. Recently biochemical analysis showed an increase in protein and lipid contents of the smooth membrane fraction from whole mouse brain treated with GSTM alone and GSTM plus SF virus A7(74) as compared to virus-infected brain only (Mehta, 1986). The importance of the availability of membranes for viral maturation may also explain why some strains of SF virus, A7(74), M103, and M9, are lethal in neonatal but not in adult mice. The cells in the brain of the neonatal mouse will be dividing, growing and differentiating and probably have an adequate proliferation of membranes for viral maturation. An induced membrane proliferation is also observed with Russian spring-summer encephalitis, St. Louis encephalitis and dengue-2 viruses (Murphy, 1980). In other virus infections such as mengovirus (Amako & Dales, 1967), vaccinia virus (Dales & Siminovitch, 1961) and poliovirus (Caliguiri & Tamm, 1970), initiation of RNA synthesis is associated with smooth membranes.

The results obtained in the present study suggest that the membrane proliferation produced by GSTM in adult mouse brain cells contributes significantly to the increased virulence of SF virus, and also possibly to that of SIN, Langat and WN viruses.

This work was supported by St. Thomas’ Charitable funds, the Multiple Sclerosis Society of Great Britain and Northern Ireland and the Philip Fleming Charitable Trust.

REFERENCES


(Received 11 June 1987)