Protection of Mice Against Bacterial Infection by Interferon Inducers

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Received for publication 17 November 1969

Antibacterial effect of known interferon inducers was investigated. Intraperitoneal injection of statolon or pyran markedly enhanced the survival of mice challenged with Klebsiella pneumoniae. The sparing effect of these two interferon inducers resembled that of bacterial endotoxin. Significant protection was obtained when the inducers were administered 24 hr before challenge. Treatments given 6 hr before or at the time of infection were ineffective. The persistence of increased resistance of treated animals to encephalomyocarditis virus strain MM, inoculated 5 days after challenge with K. pneumoniae, indicated that the bacterial infection did not adversely influence the induction or effectiveness of interferon.

Owing to the remarkably diverse nature of the many types of interferon inducers, it would seem rather unlikely that induction of interferon would be the only common biological property of these materials. Yet, a survey of publications concerning these substances revealed that, thus far, the interest in them was nearly entirely limited to their potency to induce interferon. Various bacterial endotoxins are notable exceptions in the sense that long before they were recognized as interferon inducers, they were known to induce nonspecific resistance to certain bacterial infections. After an injection of endotoxin, resistance to bacterial and viral infection appears to develop simultaneously (from the same stimulus). The purpose of this study is to report that dual resistance to infections can be also obtained after an injection of other known interferon inducers. Whether the same biological process initiated by the inducers is responsible for the increased resistance to both bacterial and viral infections, more extensive further studies can contribute to a better understanding of the intricate mechanism involved in nonspecific resistance to infection.

MATERIALS AND METHODS

Animals. Swiss albino male mice weighing approximately 25 g were used in all experiments. They were normally housed in groups of 10 per cage, with free access to commercial food and water. After challenge, they were inspected daily and the deaths were recorded.

Interferon inducers. Lipopolysaccharide B, Escherichia coli O:128:B12, referred to hereafter as endotoxin, was purchased from Difco Laboratories. Statolon, lot 354-869B-139, was kindly supplied by W. J. Kleinschmidt of the Lilly Research Laboratories. Pyran (a random copolymer of maleic acid and divinyl ether) was obtained from the Hercules Laboratories. Suspensions of the inducers were prepared in Hanks' balanced salt solution. They were inoculated intraperitoneally in 0.2-ml amounts. This volume contained 100 µg of endotoxin, 8 mg of statolon, and 200 µg of pyran, respectively.

Challenge organism. Klebsiella pneumoniae was incubated overnight at 30°C in Brain Heart Infusion broth (Difco). Well mixed undiluted culture was inoculated usually in 0.2-ml amounts intraperitoneally. This volume contained approximately 2 × 10⁶ viable cells. In some experiments, 0.3 ml was inoculated. Encephalomyocarditis virus strain MM was propagated in a continuous line of baby hamster kidney cells, as described previously (12). The inoculum was administered intraperitoneally and contained 800 plaque-forming units of virus.

Statistical evaluation. The data were analyzed by the standard methods required for chi square determinations. In all instances, the levels of significance were derived by the two-tail interpretations of the chi square values.

RESULTS

Groups of mice received single intraperitoneal injections of endotoxin, statolon, or pyran, and, on the following day, they were challenged with K. pneumoniae culture by the same route. The deaths were recorded at daily intervals for the following 10 days. The majority of them occurred during the first 2 days after challenge. Mice surviving beyond the third day retained a healthy appearance for the remainder of the observation period. The results of two representative experiments are given in Table 1. In experiment 1, the mice were challenged with 0.2 ml, and in experi-

271
ment 2 with 0.3 ml of the K. pneumoniae culture. When compared with the untreated control groups, significantly increased survival was observed not only in the groups treated with endotoxin, but also in the groups which received statolon or pyran. Chi square determinations indicated that there were no differences in the degree of protection derived from the pretreatment with statolon, pyran, or endotoxin.

The endotoxin-like behavior of statolon and pyran was observed again when groups of mice were treated with the interferon inducers and challenged with K. pneumoniae at four different time intervals. There was a direct relationship between the survival in the treated groups and the time allowed between treatment and challenge (Table 2). Significant protection was observed only in the groups treated at 24 hr before challenge (P < 0.025). When the treatments were given at 6 hr before challenge, the survival rate diminished to very nearly the same level as in the control group. Further decrease in the survival rate was associated with treatments given at the time of challenge (0 hr). Finally, when the treatments were delayed until 1 hr after the challenge, the survival rates fell below that of the control group, suggesting a deleterious effect. In all instances, the effect of both statolon and pyran approximated the effect of endotoxin.

Since the treated mice which survived the challenge with K. pneumoniae for at least 4 days remained healthy, it was feasible to rechallenge the survivors with MM virus while they still could be presumed to be refractory to this agent and, thus, to determine whether the encounter with K. pneumoniae had any effect on resistance to the subsequent viral infection. Groups of mice were treated with endotoxin, statolon, or pyran and inoculated with K. pneumoniae on the following day. After each type of treatment, the protection against K. pneumoniae was similar to that

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**Table 1. Protective effect of statolon, pyran, and endotoxin against Klebsiella pneumoniae infection**

<table>
<thead>
<tr>
<th>Treated with</th>
<th>Expt 1</th>
<th></th>
<th>Expt 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival rate</td>
<td>ps</td>
<td>Survival rate</td>
<td>ps</td>
<td></td>
</tr>
<tr>
<td>Statolon</td>
<td>30/30</td>
<td>&lt;0.001</td>
<td>35/40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pyran</td>
<td>26/30</td>
<td>&lt;0.005</td>
<td>35/40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>29/30</td>
<td>&lt;0.001</td>
<td>39/40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Control</td>
<td>16/30</td>
<td></td>
<td>11/40</td>
<td></td>
</tr>
</tbody>
</table>

*a* Level of significance of difference from control based on x² determinations. For differences between endotoxin and statolon or pyran, P > 0.05.

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**Table 2. Effect of timing of treatment with interferon inducers on survival of mice challenged with Klebsiella pneumoniae**

<table>
<thead>
<tr>
<th>Time of treatment</th>
<th>Survival rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endotoxin</td>
<td>Statolon</td>
</tr>
<tr>
<td>&lt;24 hr</td>
<td>19/20</td>
</tr>
<tr>
<td>-6 hr</td>
<td>7/20</td>
</tr>
<tr>
<td>0 hr</td>
<td>4/19</td>
</tr>
<tr>
<td>+1 hr</td>
<td>2/20</td>
</tr>
</tbody>
</table>

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**Table 3. Survival of mice treated with interferon inducers, challenged with Klebsiella pneumoniae and rechallenged with MM virus**

| Treated with | Challenged with K. pneumoniae 1 day after treatment | Rechallenged with MM virus 6 days after treatment | |
|--------------|-----------------------------------------------|-----------------------------------------------|
| Survival rate | ps | Survival rate | ps |
| Endotoxin... | 20/20 | <0.001 | 17/20 | <0.025 |
| Statolon..... | 24/27 | <0.001 | 20/24 | <0.025 |
| Pyran......... | 24/30 | <0.001 | 19/24 | <0.05 |
| Control for bacterial challenge... | 8/30 | | 5/8 | NSb |
| Control for viral challenge.... | | | | 14/27 |

*a* Level of significance of the difference from control based on x² determinations.

b Not significant.

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seen in preceding experiments (Table 3). Without any further treatment, the survivors of all groups were inoculated with MM virus on the fifth day after the challenge with K. pneumoniae (6 days after treatment with interferon inducers). In all previously treated groups, the survival was significantly higher than in a new control group which was inoculated with only MM virus. The survival in the group which was previously inoculated with K. pneumoniae only (control group for the bacterial challenge) did not differ from that seen in the virus control group. These results have indicated that the protective effect of the interferon inducers against the viral infection was not substantially diminished by the intervening bacterial infection.

**DISCUSSION**

Studies here described have shown that mice treated with statolon or pyran became markedly resistant to infection with K. pneumoniae. The degree of refractoriness to the challenge was not
significantly different \((P > 0.05)\) from that afforded by bacterial endotoxin (Table 1). Although no such properties of statolon or pyran have been reported, the increase in the resistance to various bacterial infections after administration of endotoxins has been well documented in the literature (1, 7, 14, 15). Their effectiveness has been attributed wholly or in part to induction of properdin (11). In this study, both statolon and pyran were effective only when given prophylactically; the survival rates of challenged animals were dependent on the time elapsed between treatment and challenge (Table 2). Shortening of this time resulted in decreased survival. These findings correlate with similar results of Rowley (14) and are compatible with the theory that the observed sparing effect was attributable to the activity of properdin, which was believed to exert direct cidal effect on the susceptible microorganism (11).

Statolon, pyran, and endotoxin are well recognized interferon inducers (5, 9, 17), presently best known for their induction of the antiviral state. According to a generally accepted concept, the protective mechanism of interferon against viral infection is mediated by failure of the host cell ribosomes to translate viral messenger RNA (8). Therefore, it would appear that the suppressive effect of interferon would be limited to viruses, or possibly, to those agents which are dependent on the normal function of the ribosomes of the host cell. This theory could explain the effectiveness of several interferon inducers against such nonviral but obligatory intracellular parasites as those causing trachoma-inclusion conjunctivitis (2, 16), malaria (3, 4), or toxoplasmosis (13). However, it is difficult to visualize how this mechanism could affect a bacterium such as *K. pneumoniae*, an organism fully capable of reproduction in the absence of any host cells. The properdin theory of Pillemer et al. (11) would seem to be more suitable for explanation of the results here described. Inasmuch as endotoxin is known to induce both interferon (17) and properdin (11), it appears reasonable to assume that statolon and pyran, in addition to interferon induction, can also stimulate the release of a substance related to, but not necessarily identical with, properdin. Utilization of the three inducers for protection against challenge with *K. pneumoniae* did not appear to influence the induction or effectiveness of interferon, since survivors from that infection were still resistant to rechallenge with MM virus (Table 3), to a degree comparable with the results of Kleinschmidt and Murphy (6) or of Merigan and Finkelstein (10). This finding indicates that the two protective substances may be independent of each other. The possibility of the existence of an active substance different from interferon was also proposed by Jahiel et al. in their studies of the protective effect of statolon and Newcastle disease virus against *Plasmodium berghei* (3, 4). However, an increase in the activity of other defense mechanisms resulting from administration of interferon inducers should also be considered.

**ACKNOWLEDGMENTS**

I express my sincere thanks to J. P. Schmidt for helpful review of this manuscript, to P. P. Crump for statistical analysis of the data, and to R. R. Ibarra for excellent technical assistance.

**LITERATURE CITED**


