Interferon Levels and Resistance to Viral Infection Associated with Selected Interferon Inducers (35887)

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Numerous authors have demonstrated that administration of substances known to induce interferon provokes in experimental animals a significant rise of nonspecific resistance to viral infections. According to the prevailing concept, this effect is due to interferon induced by these substances. It has been reported that the degree of protection of the intact host is directly proportional to the amount of interferon found (1). That is, the higher the interferon titer, the better the protective effect (2). We have attempted to correlate the interferon levels induced by several different substances to the degree of protection they afford, and our results show that *amounts* of demonstrable interferon have no direct relationship to protection.

Materials and Methods. Swiss albino male mice, weighing 20–25 g, were obtained from a commercial source. *Escherichia coli* 0:128:B12 lipopolysaccharide (endotoxin) was purchased from Difco Laboratories, Detroit, Michigan. Statolon, lot No. 354-1080B-21, was kindly supplied by Dr. W. J. Kleinschmidt, The Lilly Research Laboratories, Indianapolis, Indiana; maleic acid-divinyl ether copolymer (pyran) was received from Hercules Inc., Wilmington, Delaware; and polyinosinic-polycytidylic acid complex (poly I:C) was purchased from Microbiological Associates, Bethesda, Maryland. Various concentrations of the inducers, prepared in Hanks' balanced salt solution (HBSS), were administered intraperitoneally (ip) to groups of 30 mice. On the following day the mice were challenged ip with approximately 5×10^3 PFU of MM virus propagated in BHK-21 cells (3).

The protective effect of the inducers was measured by the *relative mean survival rate* (RMSR), which was calculated according to the formula: $\text{RMSR}_d = [(\sum A \times B) + d \times L]/n$, where A is the last day on which any individual mouse was alive; B is the number of mice surviving A days; d is the last day of observation (termination day of the experiment), which, in this study, was day 20 ($d = 20$); L is the number of mice which were alive on day d; and n is the original number of animals in the experimental group. The significance of the differences in the RMSR values between treated groups and untreated controls was assessed by the Student's t test (2-tailed).

Interferon titers of spleen and serum samples were determined in L cells, with BHK-propagated MM virus serving as the assay agent. Units of interferon correspond to the highest sample dilution causing a 50% plaque reduction. For circulating interferon, the animals were bled at 2 hr after administration of endotoxin or poly I:C, and at 18 hr after treatment with statolon or pyran. The assays were performed on serum pools from 10 mice. The spleens were removed immediately after the collection of blood. Pools of 10 spleens were homogenized in HBSS, using 1 ml of the fluid/spleen. Clear supernates were considered as undiluted interferon samples.

Results. The effects of increasing amounts of endotoxin and statolon on survival and spleen interferon levels are given in Fig. 1.

The increase in survival produced by 0.1 μg of endotoxin was so slight as to represent no significant difference from the control ($p > .05$). No attempt was made to determine the amount of interferon induced by this

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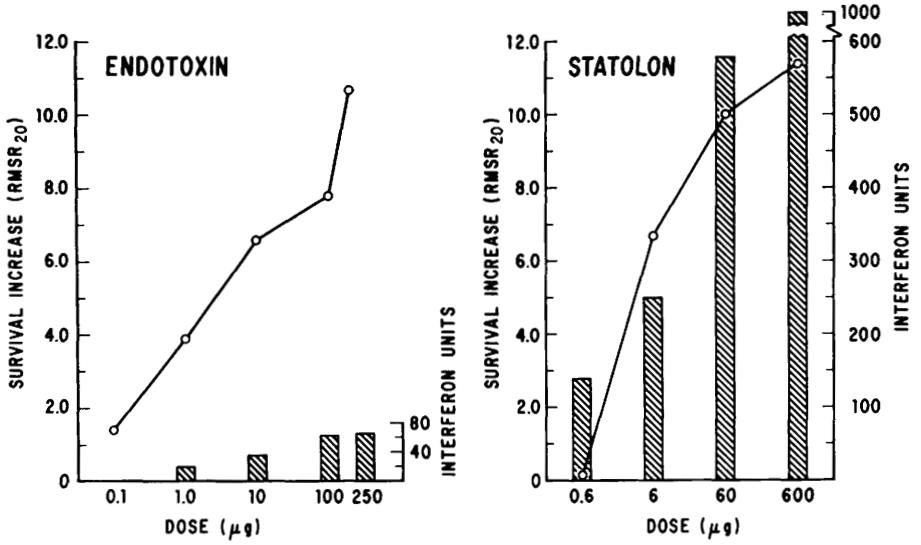

FIG. 1. Effect of endotoxin and statolon on survival (○); and interferon titer (bars) of mice challenged with MM virus.

dose. One microgram induced 20 units of interferon. The RMSR associated with this titer was significantly increased ($p < .02$). With the next three higher doses, the protective effect rose more rapidly than did the interferon levels. The highest titer, 63 units, was induced by 250 μg. In terms of survival, the doses of 10, 100, and 250 μg were increasingly effective, and the corresponding RMSR values were significant at the 1% level.

With statolon, the relationship between protection (RMSR increase) and interferon levels was different. Six-tenths microgram induced 140 units of interferon. In spite of the fact that this was more than twice the amount of interferon induced by the highest dose of endotoxin, there was no protection. The next three higher doses were significantly protective ($p < .01$), and the respective interferon titers were 250, 580, and 1000 units. For protection, 250 μg of endotoxin were very nearly as effective as 60 μg of statolon; yet, there was a striking difference in the corresponding interferon titers. For a given degree of protection, the interferon titers induced by statolon were considerably higher than those induced by endotoxin.

The serum interferon levels induced by endotoxin were slightly lower than those found in the spleen samples, while the opposite was true for the statolon-induced interferon. The differences were not considered of great consequence and, should the serum titers rather than spleen titers be used for comparisons with the levels of protection, the discrepancies would be even more pronounced.

Figure 2 shows that similar disagreements between interferon titers and protection were found when the data obtained with pyran and poly I:C were compared. The increased survival associated with 20 μg or more of pyran, and 1 μg or more of poly I:C was significant ($p<.01$). The spleen interferon levels induced by 2 to 2000 μg of pyran ranged from 8 to 27 units, and those corresponding to 1 to 500 μg of poly I:C, from 48 to 850 units. Both 200 and 2000 μg of pyran protected all animals. Complete survival was also obtained with 100 and 500 μg of poly I:C. Thus, in terms of protection, at these particular dosages the two inducers were alike, although there was no resemblance in the amounts of interferon induced. These differences would be greater yet should the comparisons be based on the serum interferon levels; the highest serum interferon induced by pyran did not exceed 10 units, while the poly I:C-induced serum titers ranged from

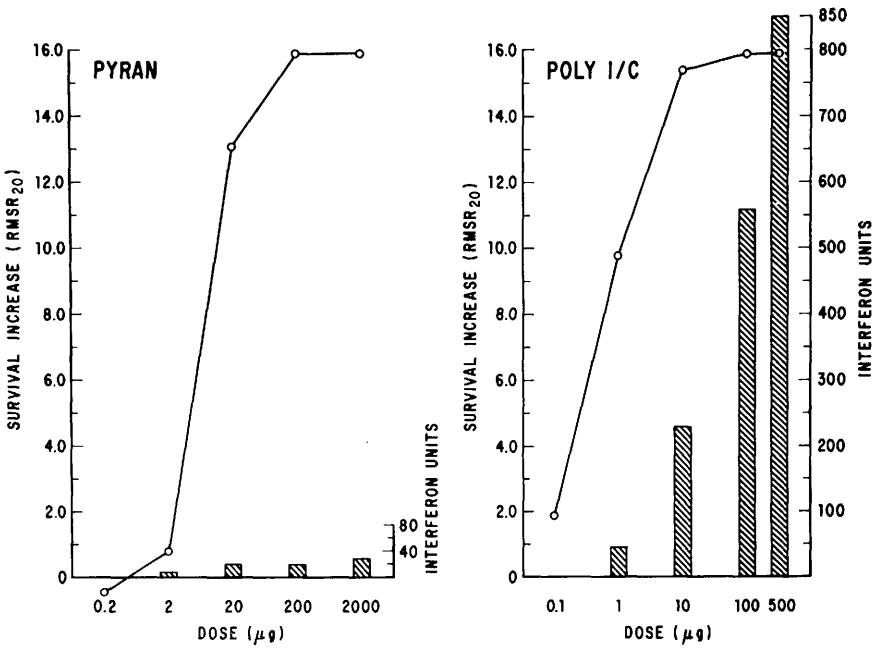

FIG. 2. Effect of pyran and poly I/C on survival (circles) and interferon titer (bars) of mice challenged with MM virus.

54 units induced by 1 μg to 15,000 units induced by 500 μg.

Discussion. Induction of interferon and protection of animals against viral infection by endotoxin, statolon, pyran, and poly I:C have been adequately documented in the literature. Interferon levels (4–6) and degree of protection (2, 5) associated with their administration have been reported to be dose-dependent. It is not clear whether the gradation of interferon response and protection against infection truly parallel each other. The mechanism of interferon action (2, 7, 8) is generally assumed to be the same regardless of the type of inducer used. Therefore, it could be expected that, in an animal, the higher the level of interferon, the greater the resistance to infection. However, reports to the contrary can be found. Thus, amounts of pyran and poly I:C which conferred nearly the same degree of protection against viral challenge could not be equated in terms of induced interferon (9). Disagreement between interferon levels and protective effect produced by poly I:C also has been reported (10).

As an extension of our own observation of certain inconsistencies between the levels of interferon and protection, we have undertaken this study for a more detailed scrutiny of the relationship between these parameters of inducer activity. We have taken certain precautions to avoid such predictable experimental errors as those caused by differences in age of animals (11), batch variations of an inducer (2), and others. All mice used in this study were from the same source, of the same strain, age, and sex, and of acceptably uniform weight. Inoculations of the inducers for determinations of both protection and interferon induction were done on the same day and from the same suspensions. All interferon samples were assayed at the same time and against the same viral agent as that used for the determinations of the resistance to infection. The interferon samples were collected at or near the time of maximal reported titers (4–6, 12, 13).

When the effect of each inducer is viewed separately, the simultaneous rise in both survival and interferon titer may suggest that the protection could be attributed to interferon. However, comparisons between inducers revealed that for approximately the

same level of protection, each inducer stimulated the release of a different amount of interferon. If protection is dependent solely on the presence of interferon, the variation in amount of interferon required for a given level of protection requires explanation. Our results suggest that, if the protection is to be fully credited to interferon, as has been proposed (1), then its protective potency depends greatly on the type of inducer used. Thus, interferon titers alone are probably rather poor indicators of the refractoriness of the host to viral infection. Materials which induce a more *potent* interferon rather than a higher titer should be sought. Alternatively, interferons induced by endotoxin or pyran may be utilized by the host in a different, more efficient way than those induced by statolon or poly I:C. Finally, the observed protection may be due to a system not directly related to interferon, as several sources have suggested (14–16). Some interferon inducers have been reported to confer protection on animals challenged with nonviral agents (17–19). Since such activity cannot be explained in terms of the mechanism of the interferon action (2, 7, 8), its presence may be only coincidental to the as yet undefined mode of protection.

These conclusions are not in agreement with those of other investigators (1). It should be noted, however, that their approach to the problem was different from ours: (i) the animals they used for interferon determinations differed in size from those used for the parallel demonstrations of protection (20 g vs 6–7 g); (ii) the assessment of these two parameters also lacked uniformity in regard to the dose of the inducer or of dextran used to demonstrate the simultaneous suppression of both interferon induction and protection; and (iii) some of the claims of significant differences appear to be subject to interpretation (in their Table II, the augmenting effect of arginine, and in Table V, survival after injection of poly I:C, with or without pretreatment with endotoxin, suggest $p > .10$).

We have demonstrated that the protection and the amount of interferon coincidental to it can be dissociated. Additional studies aimed at the differences rather than the similarities of the effect of particular inducers may eventually lead to a better understanding of the true nature of the antiviral state which follows their administration.

Summary. Comparative studies of protection of mice against infection with MM virus and levels of interferon induced by graded amounts of *Escherichia coli* endotoxin, statolon, maleic acid-divinyl ether copolymer, and polyinosinic-polycytidylic acid complex have been carried out. In every instance, both protection and interferon response were dose-dependent, but interferon titers did not correlate with protection. The degree of protection could not be correlated to any specified amount of interferon. Possible explanation of these results would include: (i) protective potency of interferon may differ with the type of inducer, (ii) different interferons are utilized for protection in different ways, or (iii) the protective effect of inducers used may be unrelated to interferon.

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