Interferon Induction by the Immunomodulating Polyanion Lambda Carrageenan

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The lambda fraction of carrageenan induced murine interferon, which was characterized by sensitivity to trypsin and inhibition by pretreatment of target cells with actinomycin D.

Recent studies have indicated that type I and type II interferon preparations have a time- and dosage-dependent immunoregulatory effect. Incubation of murine spleen cell cultures with interferon 24 h before or simultaneously with sensitization of the cultures to sheep erythrocytes (SRBC) resulted in the marked inhibition of the anti-SRBC plaque-forming cell (PFC) response (9, 12). Addition of interferon to the cultures after sensitization with SRBC enhanced the anti-SRBC PFC response (6, 13). In vivo administration of high dosages of interferon had an immunosuppressive effect, whereas lower amounts had an immunoenhancing effect (13).

One class of type I interferon inducers is characterized by high molecular weight, high density of anionic groups, and limited degradability by mammalian cells (11). Certain compounds of this class of inducers, such as dextran sulfate and pyran copolymer, also have immunomodulatory effects (2, 5). The sulfated polygalactan, carrageenan, exhibits the molecular characteristics described above and also has been shown to have both immunosuppressive (1) and immunoenhancing (E. V. Turner and R. D. Higginbotham, submitted for publication) effects on the antibody response to SRBC. In view of these similarities of carrageenan to other interferon inducers, the ability of carrageenan to induce interferon was deemed worthy of study.

All mice used in this study were 6- to 8-week-old Swiss-Webster females, and were obtained from Laboratory Supply Co., Inc., Indianapolis, Ind. Lambda and kappa carrageenan, gifts of Marine Colloids, Springfield, N.J., were suspended in saline. Appropriate dosages were injected intravenously (i.v.) and mice were exsanguinated at various time intervals as described below. Sera were assayed for antiviral activity. Control type I interferon was produced in L-929 cells by use of the Herts strain of Newcastle disease virus as previously described (8). Culture supernatant fluids were harvested, and residual inducing virus was inactivated by exposure to pH 2 at 4°C for 5 days. The antiviral activity of the preparations was determined by means of a plaque reduction assay on mouse L-929 cells using the Indiana strain of bovine vesicular stomatitis virus as the challenge virus (3). The interferon titer corresponded to the reciprocal of the highest dilution of test sample that reduced plaques by 50%. In this assay, one interferon unit is equivalent to 0.88 National Institutes of Health no. G-002-904-551 reference unit.

The antiviral activity induced by carrageenan was characterized as interferon by the following procedures. The heat sensitivity of the activity in the preparations was determined by heating at 56°C in a water bath for 1 h and then assaying for residual activity. Sensitivity to pH 2 was determined for these preparations by adjusting to pH 2 with 1 N HCl, incubating for 1 h, neutralizing with 1 N NaOH, and then assaying for residual activity. The preparations were exposed to 1 mg of trypsin per ml for 1 h at 37°C and then treated with 3 mg of ovomucoid trypsin inhibitor per ml (Sigma Chemical Co., St. Louis, Mo.) to determine trypsin sensitivity. Finally, the sensitivity to actinomycin D of the viral resistance induced by these preparations was determined. Interferon dilutions were prepared, and actinomycin D (Sigma Chemical Co.) was added to each dilution to a final concentration of 1 μg/ml. The samples were then applied to L-929 cells and incubated for 4 h at 37°C. The fluids were then removed, and the cells were washed twice to remove residual actinomycin D. Fresh medium was added, and the interferon assay was completed as described above.

Table 1 shows the titer of interferon resulting from injecting mice i.v. with either 500 or 5 μg of lambda carrageenan, doses well below those known to be toxic (14), to determine the dosage for optimum production of antiviral activity.
The mice were also bled at various time intervals to delineate the interval for production of maximum antiviral activity. The greatest activity was observed in pooled sera from mice that had received 5 \( \mu g \) of lambda carrageenan and had been bled 8 h after injection (Table 1). That this serum activity was not due to residual carrageenan was shown by the observation that treatment of L cell cultures with 5 or 500 \( \mu g \) of lambda carrageenan in medium or in normal mouse serum did not result in induction of antiviral activity. No antiviral activity was detected after injection of equivalent quantities of kappa carrageenan.

When dilutions of serum from mice bled 8 h after i.v. injection of 5 \( \mu g \) of lambda carrageenan were combined with actinomycin D, the expression of antiviral activity was inhibited (Table 2). Since the actinomycin D was dissolved in 95% ethanol, the ethanol alone was added to serum dilutions. No effect of ethanol on the antiviral activity of the serum samples was observed (Table 2). Similar inhibition of antiviral activity and sensitivity to actinomycin D were observed in Newcastle disease virus-induced interferon preparations. Neither actinomycin D (2.6 \( \times 10^7 \) PFC/ml) nor ethanol (2.6 \( \times 10^7 \) PFC/ml) inhibited the plaquing efficiency of vesicular stomatitis virus (3 \( \times 10^7 \) PFC/ml).

The interferon observed in murine serum 8 h after i.v. injection of 5 \( \mu g \) of lambda carrageenan was resistant to pH 2 treatment, sensitive to treatment at 56°C, and was inactivated by trypsin treatment (Table 3).

Several polyanions such as dextran sulfate and pyran copolymer have been shown to be inducers of interferon (4), a property shared by lambda fraction of carrageenan as shown in the present study. The antiviral activity measureable after lambda carrageenan administration appears to be due to the induction of interferon, since the activity was sensitive to digestion by trypsin and was not expressed in the presence of actinomycin D (10). This activity appears to be type I interferon due to its pH 2 stability and 56°C lability (15).

Other work has shown that "Ebimar" (Evans Medical Ltd., Liverpool, England), a degraded preparation of seaplant polysaccharide that contains both lambda and kappa carrageenan as well as other contaminating materials, induced antiviral activity (4). In the present study, lambda carrageenan but not kappa carrageenan induced interferon. Therefore, lambda carrageenan may be partly responsible for the interferon induction reported for Ebimar.

Polyanionic agents such as pyran copolymer and dextran sulfate have also been shown to have immunomodulating effects. The lambda fraction of carrageenan has been similarly shown to influence immune responses in that administration of lambda carrageenan before antigenic stimulation with SRBC caused suppression of the immune response (1) whereas administration of this fraction at the time of antigenic stimulation resulted in an enhanced antibody response (Turner and Higginbotham, submitted for publication). These immunomodulatory effects are similar to those observed with interferon (6, 9, 12, 13). The kappa fraction of carrageenan, which differs primarily from the lambda fraction by having a lesser degree of sulfation per molecule (7), has not been shown to have either immunosuppressive effects (1) or immunoenhancing effects (unpublished observations) on the antibody response to SRBC. The present study has shown that kappa carrageenan does not readily induce interferon. Therefore, the interferon induced by lambda carrageenan may contribute to the biological effects of this polyanion on immune responses.

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