Stimulation of Antibody Formation by Pyran Copolymer[1] (34433)

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Various synthetic polyanions have been shown to possess antitumor and antiviral activity. At least in the case of one of these polyanions, namely pyran copolymer (1–7), a divinyl ether–maleic anhydride copolymer of 18,000–30,000 molecular weight, this activity is associated with an induction or release of interferon in mouse and man (5, 8, 9). The interferon-inducing action of pyran copolymer, however, differs from the relatively short-lived effect produced by synthetic polynucleotides (10) in that a single injection of pyran copolymer suffices to protect animals up to 40 days from meningoencephalitis and other viruses (8). This prolonged activity, as well as the known cytotoxicity and pyrogenicity of pyran copolymer, suggested that it may act as a trigger for the release of the actual stimulators, in a manner previously indicated for bacterial endotoxins (11) which are now suspected of triggering the release of interferon (12) as well as of endogenous oligonucleotides capable of stimulating antibody formation (16). Since a number of not readily degradable polyanions, including synthetic polynucleotides such as poly A + poly U, are known to act as stimulators of both antibody formation (13) and interferon (10), we decided to evaluate the effects of pyran copolymer on antibody-forming cells in mice. The results indicate that pyran copolymer does behave in certain respects like bacterial lipopolysaccharides and synthetic polynucleotides and induces an enhancement of the early immunologic response.

Materials and Methods. Pyran copolymer NSC 46015 (pyran 3A–dicarboxylic anhydride) was provided by the Cancer Chemotherapy National Service Center in sterile 20-ml vials containing 0.1 g of the anhydride. It was stored in the refrigerator and reconstituted with water to yield a solution having a pH of 7.7 and containing 5 mg/ml of pyran. Kinetin riboside (6-furfurylaminopurine riboside, Sigma Chemical Co.) was prepared as described previously (14).

Female CF-1 mice, weighing 18–20 g, were given pyran intraperitoneally and at the same time received 10^8 sheep red blood cells (sRBC) intravenously. There were five mice in each group and the number of hemolysin-forming spleen cells per 10^8 nucleated spleen cells was assayed by the hemolytic plaque technique (15) at different times after immunization.

Results and Discussion. As indicated in Table I, the administration of pyran enhances the early response to sRBC in a manner reminiscent of the effects of polynucleotides (16) but not to the same extent. The enhancement is discernible 44 or 48 hr after immunization but not at 72 hr. The extent of enhancement varies considerably in individual animals, which is reflected in the high standard errors of the average responses in pyran-treated groups. Kinetin riboside, a known inhibitor of stimulatory effects of polynucleotides (16) also inhibits responses that occur in pyran-treated mice (Table II), in fact, it produced a less than normal response in the presence of pyran whereas in previous studies kinetin riboside given with antigen never reduced responses below normal.

Pyran effects on antibody-forming cells differ from effects produced by synthetic polynucleotides, such as poly A + poly U, in at

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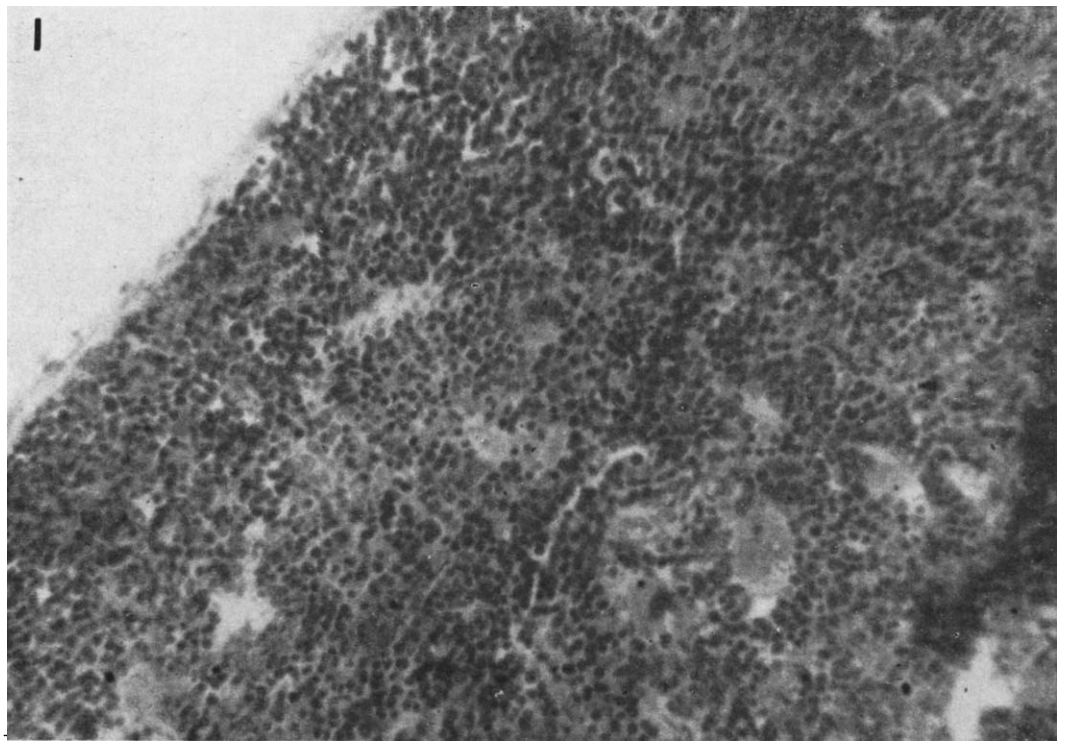

FIG. 1. Normal mouse spleen, stained with hematoxylin-eosin, 200×.

least two respects: (a) magnitude, and (b) pyrogenicity, which accompanies pyran effects but is absent in the case of poly A + poly U. In regard to pyrogenicity, which tends to occur with most cytotoxic agents, pyran resembles bacterial endotoxins (LPS) but differs from it in a very important respect: LPS, given alone, can elicit nonspecific responses, *e.g.*, can activate spleen cells that form antibodies to sRBC, and to other antigens, but pyran will not produce such nonspecific effects. Pyran thus behaves as if it were, like endotoxin and other cytotoxic agents (11, 14), a trigger for the release of stimulatory oligo- and polynucleotides but apparently it does not affect all of the cells that can be affected by endotoxin. We have previously provided evidence (17) indicating

TABLE I. Influence of Pyran Copolymer on Antibody Formation to sRBC in CF-1 Mice.[a]

Treatment of spleen donors	No. of hemolysin-forming spleen cells/10^6 nucleated spleen cells			Av spleen wt (mg) at 44 or 48 hr, respectively
	44 hr	48 hr	72 hr	
Expt. 36				
sRBC (10^8)	257.7 ± 69.1		7895 ± 223	109
+ pyran (2500 μg)	873.0 ± 114.1		6435 ± 109	223
Expt. 43				
None		13.4 ± 0.7		108
sRBC (10^8)		422.7 ± 48.2		114
+ pyran (2500 μg)		826.3 ±121.7		254
Pyran (2500 μg)		20.1 ± 0.6		134

[a] Pyran was given ip. Five mice/group.

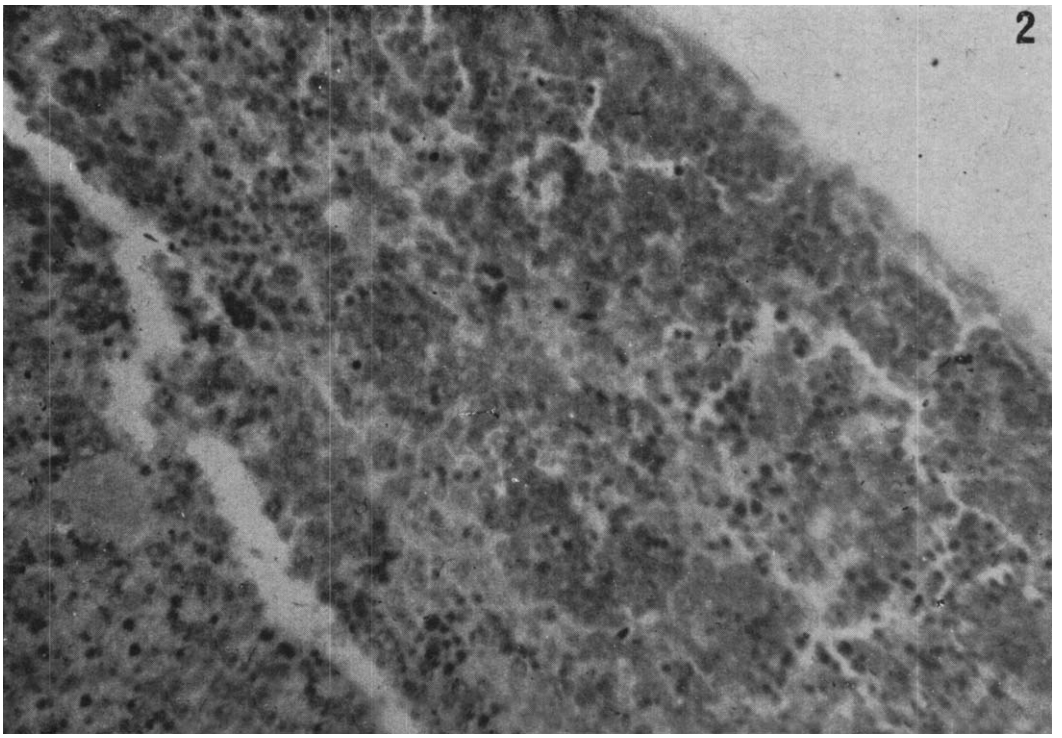

FIG. 2. Mouse spleen 24 hr after intraperitoneal injection of 125 mg/kg of pyran copolymer, stained with hematoxylin-eosin, 300×.

that the nonspecific initiation of antibody formation elicited by endotoxin may occur at the level of lymphocytes. A stimulation of specific antibody response, however, can be the consequence of stimulatory effects on either lymphocytes or macrophages (16, 18, 19). One could therefore suspect that pyran affects, principally, events at the level of phagocytes, and that its lesser stimulation of antibody formation, compared to the effects of polynucleotides, is due to an effect on only one component of a multicomponent system.

TABLE II. Influence of Pyran Copolymer and Kinetin Riboside on Antibody-Formation to sRBC.

Treatment of spleen donors	No. of hemolysin-forming spleen cells/10^8 nucleated spleen cells 48 hr after immunization
sRBC	433.9 ± 33.0
+ pyran	623.7 ±119.0
+ pyran + kr	152.6 ± 22.5
Pyran	45.3 ± 4.4
None	22.2 ± 3.1

The conclusion that pyran principally affects phagocytic cells is supported by cytologic evidence concerning changes in the RES following the intraperitoneal administration of pyran copolymer to mice. Figures 1–3 show that within 24 hr after pyran injection the small cells of the germinal centers disappear and the spleen becomes loaded with large pyroninophilic reticulum cells of the kind associated with enhanced phagocytosis. The influence of pyran on phagocytic events is further indicated by the appearance, in fixed histocytes of pyran-treated animals as well as in the circulation, of monocytes loaded with basophilic particulates, apparently due to an incorporation of pyran into the cytoplasm as a function of phagocytic activity (9). The spleen weight data in Table I reflect the known capacity of pyran to induce splenomegaly.

Pyran copolymer, which is currently under study as an antitumor agent and an inducer of interferon, may thus be of some value in other systems in which a principal effect on

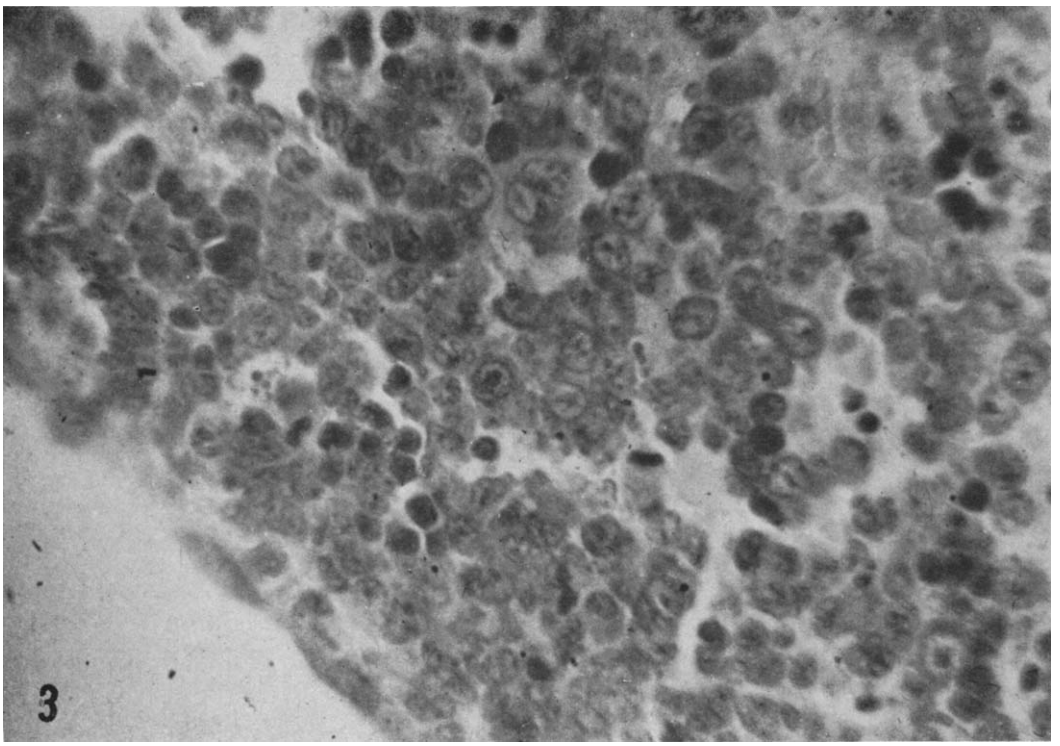

FIG. 3. Same as Fig. 2 but at higher magnification showing depletion of small lymphocytes and pyroninophilic cellular reaction; stained with methyl green-pyronin, 800×.

macrophage-associated events is desired, including a stimulation of early events in immune responses.

In regard to the mechanism of action, it would appear, on the basis of the results with kinetin riboside (a known inhibitor of stimulatory oligonucleotide effects) that the effects of pyran on phagocytic events are not direct but a consequence of pyran's ability to release, by cytotoxicity, a stimulatory material from intracellular sites. This material may be oligonucleotide in nature, since it is known (a) that oligonucleotides derived from natural polynucleotides (20) or synthetic polynucleotides (16, 21) can stimulate phagocytosis, and (b) that such effects are inhibited by kinetin riboside. Pyran thus may serve as the trigger for the release of the actual stimulators of immunologic activity.

Finally, in applying pyran as a stimulator of macrophage activity, it should be kept in mind that the effects of this polyanion are known to be dependent on route of administration and on time of administration in relation to antigen (22) since, as in the case of bacterial lipopolysaccharides, pyran can have a biphasic effect on the phagocytic activity of the RES.

Summary. Pyran copolymer, administered with sheep red blood cells to mice, enhances the early rate of appearance of antibody-forming spleen cells. Although the cytotoxicity and pyrogenicity of pyran and bacterial endotoxins are similar, pyran, in contrast to endotoxin, does not trigger a nonspecific initiation of antibody formation; it only enhances specific responses. The major effect of pyran appears to be on macrophages.

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