Immunosuppression, Interferon Inducers, and Leukemia in Mice (34783)

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(Introduced by F. A. Murphy)

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Various polyanions can induce interferon production or release in experimental animals and man (1–3). These agents are also capable of preventing virus-induced leukemias and sarcomas in rodents (2–5) offering hope that they may afford similar protection against any possible virus-induced malignancies of man.

An area of great potential usefulness of these interferon inducers is in the prophylaxis against viral infections in humans whose cell-mediated immunologic defense mechanisms are compromised by disease or by immunosuppressive chemotherapy. Experimental animals subjected to neonatal thymectomy or treated with immunosuppressive agents such as antilymphocytic serum (ALS) have been found to have an enhanced susceptibility to infection by the non-oncogenic viruses such as vaccinia (6), herpes simplex (7), and canine distemper (8) as well as to oncogenesis following infection by such agents as polyoma (9–11) and leukemogenic viruses (10, 12). Humans whose cell-mediated immune mechanisms are depressed have similarly been found to be increasingly susceptible to herpes simplex (13) and vaccinia viruses (14), as well as to measles (15), varicella-zoster (16), and cytomegaloviruses (13, 17). Such patients have also been reported to have a markedly increased incidence of neoplasms, possibly virus-induced, particularly of the lymphoreticular systems (18, 19).

It was therefore of interest to study whether severely immunosuppressed animals could respond to interferon inducers and could be protected by them against virus-induced leukemias and lymphomas. The most satisfactory method of producing profound and prolonged supression of cell-mediated immunity in the mouse is by adult thymectomy followed by short-term administration of ALS (20, 21). This report describes the effects of pyran anionic copolymer on the course of Rauscher leukemia virus infection in both severely immunosuppressed and normal mice.

Materials and Methods. C_3H/HeJ and A/He male mice weighing approximately 25 g were obtained from Jackson Laboratories, Bar Harbor, Maine. Four-week-old randomly bred male Swiss mice were obtained from Charles River Farms, Boston, Mass. Rauscher leukemia virus (RLV) in a 10% spleen extract was provided by Dr. Frank Rauscher, National Institutes of Health, Bethesda, Maryland. Pyran copolymer (NSC-46015; pyran-2-succinic anhydride, 4,-5-dicarboxytetrahydro-6 methyl-, anhydride polymers) was provided by the Cancer Chemotherapy National Service Center, Bethesda, Maryland.

Rabbit antimouse lymphocyte serum was prepared as described previously (22). New Zealand rabbits were injected in the footpads with 100×10^6 A/He lymph node lymphocytes in a saline suspension which was emulsified with an equal volume of complete Freund adjuvant (Difco). Three weeks later booster injections of 100×10^6 lymph node lymphocytes in saline suspension were given intravenously on 3 successive days and the rabbits were bled from the ear artery 1 week after the last booster injection. The immunosuppressive effect of the serum was tested by injecting A/He mice with 0.5 ml of ALS ip on days -1 and $+2$ in relation to placement of first set C_3H/HeJ skin grafts. The median survival time (MST) $\pm$ standard error (SE) of skin grafts in the ALS-treated group was 34.0 ± 2.8 days compared to a normal MST of 10.2 ± 0.3 days in this strain combination.

The design of the experiments performed is

TABLE I. Effects of Pyran Copolymer on Rauscher Virus Leukemia in Normal and Immunosuppressed C_3H Mice.

Group	No.	Thymectomy (day −10)	ALS (days −8 and −6)	Pyran (days −5 to −1)	Rauscher virus (day 0)	Mean spleen wt[a] ± SE (g)	Reduction in virus titer[b] (logs)	Interferon titer (units/ml)
A	14	+	+	+	+	0.151 ± 0.011	>2	40
B	14	+	+	—	+	0.416 ± 0.078	0	31
C	20	—	—	—	+	0.349 ± 0.022	0	19
D	21	—	—	+	+	0.176 ± 0.009	>2	39
E	12	—	—	+	—	0.183 ± 0.007	—	24
F	7	+	+	+	—	0.147 ± 0.013	—	40
G	12	—	—	—	—	0.086 ± 0.004	—	—
H	9	+	+	—	—	0.090 ± 0.003	—	—
I	14	—	+	—	—	0.110 ± 0.005	—	—

[a] Spleen weights measured on groups of 6–14 animals 6 weeks following infection; mean spleen weights of groups B and C were significantly greater ($p < 0.05$) than those of groups A and D, respectively; mean spleen weights of groups E and F were significantly greater ($p < 0.05$) than those of groups G and H, respectively.

[b] Virus titers in blood at day +21 determined by bioassay in 4-week-old Swiss mice. Reduction in virus compared with stock RLV pool which titered $10^{3.0}$ SED_{50}/ml in nonimmunosuppressed Swiss mice.

shown in Table I. Thymectomies were performed in animals of groups A, B, F, and H at 8 weeks of age (day −10 in relation to subsequent virus infection) according to techniques previously described (20). On days −8 and −6 groups A, B, F, H, and I received 0.5 ml of ALS intraperitoneally. Pyran copolymer, dissolved in isotonic saline, was given in daily intraperitoneal doses of 25 mg/kg of body weight to groups A, D, E, and F on days −5 to −1. On day 0 groups A, B, C, and D were inoculated intraperitoneally with 100 SED_{50} (50% spleen enlarging doses in BALB/c mice) of RLV.

Blood was collected for interferon analysis on day 2 from animals of groups A–E and the specimens from within a group were pooled. Sera were acidified (pH 2) for 48 hr, brought back to neutrality, and tested for interferon activity using a vaccinia virus assay described by Lindenmann and Gifford (23) and modified for use with mouse embryo fibroblasts. The reciprocal of the dilution giving 50% inhibition was used to represent the units of interferon per unit volume of serum.

Blood was collected on day 21 from groups A–E, pooled, and titrated for virus according to the bioassay method of Chirigos (24). Serial serum dilutions were inoculated ip into 4-week-old male Swiss mice recipients; 21 days later the recipient animals were killed and their spleens were weighed to the nearest milligram.

Animals of all groups (A–I) were examined twice weekly for splenomegaly. On day 42, several animals (6–14) from each group were sacrificed and their spleens were weighed; representative spleens from each group were fixed in buffered formalin and sections were stained with hematoxylin and eosin. The remaining animals were followed until death.

Results. Treatment of mice with pyran copolymer prevented the development of Rauscher virus-induced leukemia, both in normal and severely immunosuppressed animals (Table I). Spleens first became palpable in immunosuppressed mice infected with Rauscher virus (group B) during the fourth week following virus inoculation, and in nonimmunosuppressed animals (group C) 6 weeks after infection. Spleens were not palpable in any other groups when several animals from each group were sacrificed 6 weeks following infection. At that time the mean spleen weights of infected pyran-treated groups, both immunosuppressed and normal (groups A and D, respectively) were significantly lower than the mean spleen weights of the matched nonpyran groups (B and C)

($p<.05$, using a multiple comparisons procedure based on 99% confidence limits constructed about the means). Pyran treatment alone increased spleen weights slightly in both uninfected normal and immunosuppressed animals (groups E and F, respectively, $p<.05$), but no significant differences were found between these groups and those groups that received RLV in addition to pyran copolymer (A and D).

Histological examination of spleens from sacrificed animals of nonpyran-treated infected animals (groups B and C) showed characteristic erythroblastic Rauscher virus leukemia. No such changes were observed in spleens of infected pyran-treated groups (A and D). Depletion of splenic small lymphocytes was observed in pyran-treated immunosuppressed animals (group A), whereas spleens from nonimmunosuppressed pyran-treated animals infected with RLV (group D) showed hyperplasia of lymphocytes and reticuloendothelial cells.

Animals that were not sacrificed at 6 weeks have now been followed for 3 months. In the remaining animals palpable spleens have been noted in 1 of 7 animals in group A, 3 of 5 in group B, 7 of 13 in group C, and 1 of 11 in group D.

Blood virus titers measured in various groups 21 days after infection, corresponded to subsequent spleen weights and histologic evidence of leukemia in the groups tested (Table I). Pyran-treated groups had virus titers in serum 2 to 3 logs lower than their matched groups not treated with pyran.

Immunosuppressed animals appeared capable of producing interferon when stimulated either by pyran copolymer or by RLV. No significant differences were found in serum interferon titers between normal and immunosuppressed animals or between pyran-stimulated and unstimulated RLV-infected mice (Table I).

Discussion. An unusually high incidence of lymphoreticular malignancies has been noted in human transplant recipients (18, 19), and in patients with immunologic deficiency disorders, such as ataxia–telangiectasia (25) and Wiskott-Aldrich syndrome (26). These groups share the characteristics of having profoundly depressed cell-mediated immune systems and enhanced susceptibility to viral infections. Whether the malignancies in these patients are virus induced is not known. However, numerous animal model systems exist, both planned and accidental, of immunosuppression followed by virus-induced maligancies (9–12, 27). In contrast, animals treated for long periods of time with immunosuppressive therapy but not exposed to extraneous oncogenic viruses do not appear to have an increased incidence of neoplasms [(11); and Monaco, A. P. and McDonough, E., Jr., unpublished data]. These observations suggest that the increased oncogenesis observed in immunosuppressed patients may also be dependent upon decreased immunologic responsiveness to oncogenic viruses.

One approach to the problem of viral infections in immunosuppressed hosts is to stimulate the production of interferon. The present study indicates that profoundly immunosuppressed mice can respond both to pyran copolymer and Rauscher leukemia virus with the production of interferon. No significant differences in circulating interferon titers could be found between immunosuppressed and normal groups although the times that sera were tested (2 days after infection) may have been too late to determine early differences. Barth *et al.* (28) have recently shown that ALS can transiently depress the interferon response in mice to another polymer (poly I:C) and to Newcastle disease virus, although the depressed animals could still produce 2000–2500 units of circulating interferon.

The mechanism of protection afforded by pyran copolymer in the present study may not have been via increased interferon production. The observation that virus titers in spleens of pyran-treated infected mice were 2–3 logs lower than titers in infected mice not treated with pyran does suggest that some antiviral effects were operative. In addition to interferon, these may have been enhanced phagocytosis, increased circulating antibody, or accelerated elimination of virus-infected cells by cell-mediated immune mechanisms. All of these mechanisms of host resistance have been reported to be enhanced in poly-

mer-treated animals [(29); and H. B. Levey, unpublished observations]. It is unlikely that stimulation of either cell-mediated immunity or circulating antibody could account for the protection offered by pyran since thymectomy plus ALS has been shown to profoundly depress these mechanisms (20, 21). In addition, spleens of immunosuppressed animals treated with pyran showed no repopulation of small lymphocytes on histological examination. Furthermore, pyran does not shorten survival of A/He skin grafts on C_3H mice who have been thymectomized and treated with ALS (unpublished observations). Although the precise target cell of pyran action is not fully understood, reticuloendothelial cell hyperplasia observed in both normal and immunosuppressed animals suggests that at least part of the protection against viral leukemogenesis may involve enhanced phagocytosis.* Further studies on the protection provided by pyran copolymer are in progress.

Both pyran copolymer and Poly I:C have been found to be capable of inducing interferon in man (3, 30), although both are also pyrogenic. These polymers have also been shown to have considerable toxicity in experimental animals (31, 32). Work is in progress in several laboratories to produce more potent and less toxic interferon inducers, and recent reports indicate such polymers are becoming available (33). Perhaps these less toxic agents may prove useful in the prevention of viral infections and possible viral oncogenesis in immunosuppressed patients such as those undergoing organ transplantation.

Summary. An interferon-stimulating compound, pyran copolymer, has been shown to protect adult thymectomized mice treated with antilymphocyte serum from Rauscher virus-induced leukemia without affecting their unresponsiveness to skin homografts. Although the mechanisms of this protection are uncertain, severely immunosuppressed hosts have been shown to be capable of producing interferon following pyran copolymer challenges. The potential usefulness of these agents in the prophylaxis against virus diseases and possible virus oncogenesis in immunosuppressed humans is discussed.

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* Since submission of this manuscript, Braun, W. *et al.* (Proc. Soc. Exp. Biol. Med. 133, 171 (1970)) have demonstrated that pyran is a potent stimulator of macrophage activity.

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