Suppression of Experimental Allergic Encephalomyelitis by Tilorone: Cell Transfer and Interferon Studies

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Abstract: *Tilorone is known to induce interferon and to suppress experimental allergic encephalomyelitis (EAE) produced by active immunization. In the present work, tilorone was given to donors or recipients of the passive transfer form of EAE, using a localized variety of 1 or 4 days duration and a nonlocalized form of 8 days duration. The results indicated that tilorone inhibited EAE by retarding the clonal expansion in response to antigen, by a reversible inhibition of EAE effector cells, and probably by an effect on nonspecific reactive cells in the perivascular inflammatory infiltrates. Inhibition of EAE was not mediated by the induction of interferon, but was associated with depletion of T lymphocytes.*

Key Words: Allergic encephalomyelitis; Tilorone; Interferon; Antilymphocyte serum; Immunosuppression

INTRODUCTION

Tilorone, an immunosuppressive drug that also induces interferon (Levin and Albrecht, 1981), is of particular interest for the treatment of T lymphocyte-mediated autoimmune diseases because it depletes these cells selectively from all the peripheral lymphoid tissues (Levine et al., 1974; Levine, 1977). Antilymphocyte serum (ALS) has a similar, but not identical, lympholytic effect and the two agents interact synergistically (Levine et al., 1983). Megel et al. (1974, 1977) showed that tilorone ameliorated the development of experimental allergic encephalomyelitis (EAE) produced by active immunization with spinal cord antigen and complete Freund's adjuvant. Tilorone had additive suppressive effects on EAE with several other drugs and a synergistic effect with cycloleucine (Levine and Sowinski, 1977). However, the drug was like a double-edged sword, because under certain conditions tilorone caused generalized tremors, spinal cord necrosis, formation of nuclear bodies in astrocytes, or relapse after apparent recovery from EAE (Levine, 1977; Levine and Sowinski, 1976; Levine et al., 1975). We have pursued the mechanism of immunosuppression by tilorone in the hope of separating the useful from the undesirable components of its complex activities. In the present work, we have dissected some of the cellular mechanisms by producing EAE by passive transfer of living lymphoid cells from actively immunized donors to immunologically naive recipients. Because

Received November 8, 1982; revised and accepted January 13, 1983.

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52 Vanderbilt Ave., New York, NY Immunopharmacology 6, 23–30 (1983) 0162-3109/83/$03.00

the immunopathologic reaction in the localized variety of passive EAE is histologically visible in so short a period as one day, we have been able to follow the time course of the immunosuppression and yet avoid many of the pharmacologic and pathologic complexities mentioned above.

MATERIALS AND METHODS

Lewis rats from Harlan Sprague Dawley Inc, Walkersville, MD, were maintained, in hanging wire cages, on Purina Laboratory Chow and tap water. Tilorone (2,7-bis-(diethylaminoethoxy) fluoren 9-one) hydrochloride was dissolved in saline and administered without anesthesia by gastric tube, except as noted otherwise. Rat interferon was prepared as described previously (Abreu, 1982; Lin and Abreu, 1979), stored frozen, and injected intravenously as soon as it was thawed. ALS was a rabbit antirat lymphocyte preparation purchased from M.A. Bioproducts.

Passive transfer of EAE was done with female, 150–200g, Lewis rats as donors and male, 250–350g, Lewis rats as recipients. EAE was produced in the donors by injecting the right footpad with 0.05 ml of an emulsion of 40% guinea pig spinal cord homogenate and an equal volume of Freund's complete adjuvant (Bayol–Arlacel, 8.5:1.5, plus 4 mg/ml killed tubercle bacilli). Pertussis vaccine concentrate (0.1ml, 20 billion organisms) was inoculated into the dorsum of the same foot as an ancillary adjuvant. 7 or 8 days later, the draining lymph nodes were harvested, processed into a cell suspension, and washed, and one donor equivalent was injected intravenously into each recipient rat (0.5 equivalent for the ALS experiments.)

For the localized form of EAE, the recipients were prepared 3–6 days before the anticipated killing time with a thermal injury of the right cerebral hemisphere. The scalp was incised under ether anesthesia and a preheated electric soldering iron was applied for 7 sec to the intact calvaria. The heat produced a large cup-shaped zone of necrosis in the underlying brain but it caused no detectable abnormality in the rats' behavior. The rats were usually killed 1 or 4 days after passive transfer. Brains were fixed in Bouin's fluid. Four or five frontal slices, including the entire necrotic area, were embedded in paraffin and sectioned and stained with hematoxylin and eosin; for details and illustrations, see Levine and Hoenig (1968). EAE scores were based on the density of EAE lesions on the perimeter of the necrotic lesions. Rats scored 4+ had lesions along virtually the entire perimeter, whereas 1+ implied from 1–5 widely scattered lesions on the entire slide, and 2+ and 3+ were intermediate. Slides were randomized and scored without knowledge of whether EAE cells had been given, or their number or type. For some experiments, passive transfer was also done without any localizing procedure. Instead of killing after 1 day, the rats were followed for 8 days for clinical signs of EAE: atonic, paralyzed tail (1+); hindlimb weakness (2+); or paralysis (3+). For histologic confirmation, the entire spinal cord was studied for perivascular infiltrates and scored from 1+ to 4+ in a manner analogous to the scoring of the localized form.

In some experiments, serum was obtained at time of killing. It was assayed for interferon as described previously (Abreu et al., 1981).

RESULTS

Tilorone Treatment of Recipients

Control rats killed one day after passive transfer of EAE cells had lymphocytic perivascular infiltrates in the brain around the perimeter of the thermal injury, constituting the localized form

Abbreviations. EAE: experimental allergic encephalomyelitis; ALS: antilymphocyte serum.

of EAE. The infiltrates were numerous, warranting scores of 4+ except for a few rats. Rats that were given tilorone by gavage almost invariably had fewer infiltrates. The inhibition of EAE was dose-dependent. The drug was equally effective when given shortly after the EAE cells or on the previous day, but it was somewhat less inhibitory when given 3 or 6 days before the EAE cells (Table 1). The effectiveness of prior treatment with tilorone has also been noted in EAE produced by active sensitization (Megel et al., 1977).

Tilorone is known to induce interferon with peak values after 1 day (Rhoads et al., 1973). The rats killed one day after treatment with EAE cells and 100 mg/kg tilorone had serum interferon titers of 8–64 units/ml. However, the groups of rats given doses of 20 mg/kg on the day of cell transfer or one day earlier, or doses of 100 mg/kg 1 or 3 days before cell transfer, did not have detectable interferon despite the inhibition of EAE found under each of these conditions (Table 1). The same was true of a group given 70 mg/kg tilorone through the drinking water (not included in Table 1). Thus, there was no correlation between interferon levels and inhibition of EAE in these passive transfer experiments.

In a further experiment (not included in Table 1), 3 rats were given 100 mg/kg tilorone 3 days before EAE cells and a second identical dose on the day of cell transfer. Pretreatment with tilorone is known to induce hyporeactivity to a subsequent dose (tachyphylaxis) (Stringfellow and Glasgow, 1972). As expected, tachyphylaxis reduced the interferon titer to 4 u/ml in each of these rats, compared to 64 u/ml in each of 3 rats that received tilorone only on the day of cell transfer. Nevertheless, inhibition of EAE was equally severe in both groups (average score 0.3 in each group). In a final, but unsuccessful, attempt to find a link between tilorone's inhibition of EAE and interferon, single suboptimal doses of 5,000–10,000 units of purified rat interferon were given to 5 groups of 3 rats at the same time, or one day before or after the administration of 20 or 100 mg/kg tilorone. None of these groups given the combination treatment had more inhibition of EAE or higher titers of interferon than that attributable to the tilorone alone.

Tilorone and ALS both deplete T lymphocytes from peripheral lymphoid tissues and the combination is more effective than either agent alone (Levine et al., 1983). The combination also shows additive suppression of EAE produced by active immunization (Levine and Sowinski, 1977.) If T lymphocyte depletion is an important component of tilorone's immunosuppressive activity, such an additive effect ought to be demonstrable in the passive transfer system also. Therefore, several experiments were done to establish dose levels for each of two batches of

Table 1 *Inhibition of the passive localized form of EAE by tilorone treatment of recipients*

	Localized EAE, average histologic score			
		Day of treatment[a]		
Tilorone dose mg/kg	*Day −6*	*Day −3*	*Day −1*	*Day 0*
200		2.7±1.1 (6)	0.8±0.8 (3)	1.7±0.5 (3)
100	2.4±0.7 (8)	1.1±0.6 (7)[b]	0.4±0.5 (5)[b]	1.2±1.2 (17)[c]
20			1.7±0.5 (3)[b]	2.1±0.7 (16)[b]
10				2.7±0.5 (3)
2				4.0±0 (3)
0				3.4±0.8 (39)

[a]Tilorone administered by gavage to recipients, 6, 3, or 1 day before passive transfer of EAE cells on Day 0, or within 1 hr after transfer on Day 0. Rats were killed 1 day after transfer. Scores ± SD (average, scale 0–4 +) represent lesions adjacent to thermal injury. The number of rats are in parentheses. Twelve experiments are summarized.

[b]Serum on Day + 1 did not have detectable interferon.

[c]Serum on Day + 1 had 8–64 units/ml of interferon.

ALS that would partially inhibit passive localized EAE after administration to recipients. When combined with a suboptimal dose of tilorone, there was a clear additive suppressive effect (Table 2). This finding supports a role for T lymphocyte depletion in tilorone's suppression of EAE.

The duration of tilorone's inhibitory effect was studied in rats that had thermal injuries inflicted 1 or 4 days after simultaneous injection of EAE cells and treatment with tilorone. These groups of 4 rats were killed 4 or 7 days, respectively, after cells and tilorone (i.e., 3 days after the thermal injury), so that the EAE cells had 2 days to produce perivascular infiltrates (the perimeter of the thermal injury is not hospitable to EAE cells for its first 24 hrs (Levine et al., 1968)). Tilorone treatment caused considerable inhibition of passive localized EAE in rats killed after 4 days (average histologic score 1.0, compared to 3.0 for controls given cells but no drug) and also after 7 days (average score 1.0, compared to 3.3 for controls.) However, tilorone administered at the same time as the passive transfer could damage both the donor EAE cells as well as the recipient's nonspecific reactive cells. This experiment confirmed the prolonged inhibitory effect of tilorone noted in Table 1and already reported for actively induced EAE (Megel et al., 1977), but it could not contribute to determining tilorone's cellular target(s).

Tilorone Treatment of Donors

In all of the previous experiments, tilorone administered to recipients had an opportunity to interact with the specifically sensitized EAE cells transferred from the donors, as well as with the recipients' nonspecific reactive cells, whether it was given before or after the passive transfer. This is due to the drug's persistence in the tissues (Hook et al., 1974). In order to identify the cellular target(s) of the drug treatment, tilorone was given to the donors during the active sensitization period. Their lymph node cells were procured 7 or 8 days after immunization and injected into recipients in the usual way. In order to study their fate, each of the following experiments included groups of recipient rats with thermal injuries that were killed 1 or 4 days after passive transfer, as well as groups of recipient rats (without thermal injuries) that were followed for 8 days to permit development of the conventional (nonlocalized) form of EAE. The latter occurrence was monitored by development of both clinical signs and histologic lesions, unlike the localized form which was manifested by histologic lesions only.

Table 2 *Additive inhibition of passive localized EAE by treatment of recipients with tilorone and antilymphocyte serum (ALS)*

Treatment[a]		
Tilorone mg/kg	*ALS ml/kg*	*Localized EAE score, histologic*[b]
—	—	2.3 ± 0.6
20	—	1.3 + 0.9
—	0.4	1.4 ± 0.6
20	0.4	0.4 + 0.4
—	—	2.4 ± 0.6
20	—	0.8 ± 1.0
—	0.8	1.0 ± 0.5
20	0.8	0.3 ± 0.4

[a]Tilorone by gavage and ALS by intraperitoneal injection right after EAE passive transfer. ALS batches of slightly different potencies were used for the two experiments.

[b]Average ± SD (scale 0–4 +) of groups of 7 rats killed 1 day after passive transfer. EAE in untreated controls is lower than in Table 1 because only 0.5 donor equivalents were given to each recipient.

Table 3 *Inhibition of the passive localized and nonlocalized forms of EAE by tilorone treatment of donors*

	Donors			Recipients			
Tilorone	EAE	Lymph	Cell	Localized EAE		Nonlocalized EAE after 8 days	
treatment[a]	signs[b]	nodes[c]	count[d]	One day[e]	Four days[e]	Clinical[f]	Histologic[g]
None	20/20	0.31g	2.5×10^8	3.7	ND	5/5	4.0
None	8/8	0.32	2.2×10^8	ND	ND	8/8	ND
Saline	13/17	0.34	ND	3.7	ND	3/3	4.0
Day 0	1/23	0.24	1.2×10^8	1.5	1.0	0/5	0.8
Day 0	1/16	0.22	0.8×10^8	1.3	0.6	0/8	0.3
Day +6	17/20	0.30	2.7×10^8	2.0	3.3	2/5	2.0
Day +7	24/30	0.39	4.5×10^8	1.5	4.0	5/5	ND
Day +7	14/23	ND	ND	0.2	ND	5/5	2.7

[a]200 mg/kg by gastric tube on day of immunization (Day 0) or indicated day thereafter. Each line is a separate experiment.

[b]Numerator is number of donor rats with clinical signs of EAE when lymph nodes were harvested 7 or 8 days after immunization. Denominator is total number of donor rats.

[c]Wet weight donor of nodes draining site of inoculation. ND = not done.

[d]Mononuclear cell count/donor of suspension made from lymph nodes. ND=not done.

[e]Average score (scale 0–4+) of lesions adjacent to thermal injury in recipients, 1 or 4 days after cell transfer. There were 3–6 rats in each group.

[f]Incidence of weakness or paralysis in recipients without thermal injuries.

[g]Average score (scale 0–4+) of lesions in spinal cord, 8 days after cell transfer.

Almost all non-drug-treated (control) donor rats had clinical signs of EAE when killed 8 days after active immunization. They provided 0.31–0.35g (wet weight) of lymph nodes and 2.2 to 2.5×10^8 of lymph node cells from each donor (first three experiments of Table 3). In response to injection of these control cells, recipient rats with thermal injuries developed numerous EAE lesions adjacent to the thermal necrosis, exactly as described previously. Other rats without thermal injuries developed clinical signs 4 days after injection of EAE cells. The signs usually progressed to paralysis and were attended by numerous perivascular inflammatory lesions in spinal cord.

In all the remaining experiments detailed in Table 3, 200 mg/kg tilorone was administered by gavage to prospective donor rats. When the tilorone was given on the same day as immunization with spinal cord antigen and adjuvants, only 1 of many donors had clinical signs after 7 or 8 days, due to the immunosuppressive effect of the tilorone treatment (the others would have had signs on day 9 or 10, as indicated by cohorts that were kept alive in order to determine the length of the delay). The yield of lymph node cells was reduced in both experiments. Recipients of these cells, killed 1 or 4 days later, had relatively few lesions adjacent to the thermal necrosis. Furthermore, other recipients without thermal injuries did not develop clinical signs even after 8 days, and their spinal cords developed only minor lesions. An additional group (not included in Table 3) was followed until 11 days after the transfer, but there was still no evidence that the donor cells had recovered any encephalitogenic potency.

Tilorone was given 6 or 7 days after immunization in three experiments, a time when most donors already had clinical signs of active EAE. When the donors were killed the next day, the drug had not retarded the progression of clinical signs or retarded the development of new signs in the other donors, nor was there any decrease in the yield of lymph node cells. Nevertheless, the encephalitogenic capacity of these cells to produce localized EAE was impaired in rats killed

after 1 day, albeit to different degrees in the three experiments. Localized EAE after 4 days and nonlocalized EAE after 8 days exhibited relatively little impairment, indicating that the additional time enabled the donor cells to recover from the tilorone treatment (Table 3).

These results might also be explained by carryover of tilorone, bound to the donor lymph node cells, sufficient to inhibit localized EAE in 1 day, but too little to persist 8 days in the nonlocalized system. However, the data of Table 1 show that a dose of 10 or 20 mg/kg was required for a moderate degree of inhibition of localized EAE, and it was hardly likely that washed cells from 0.39 g of lymph nodes could contain 5–10% of the entire 200 mg/kg dose of tilorone given to a 200 g rat. For experimental verification, part of the suspension of EAE lymph node cells from the last experiment in Table 3 was heat-killed at 60°C for 20 min and then stored frozen. Several weeks later, thawed cells derived from one donor were injected into each of 3 recipient rats 1 hr after these rats had also received a living lymph node cell suspension. When killed the next day, each rat had 4+ scores for localized EAE, equivalent to controls that received only the living cells. Thus, there was no detectable immunosuppression that might be attributed to carryover of tilorone.

Suppressor Cells

Inhibitory effects of tilorone given to donors or recipients might be due to activation of nonspecific suppressor cells. This possibility was tested by intravenous injection of recipient rats with 3.8×10^8 spleen cells taken from unimmunized rats that had been treated with 100 mg/kg tilorone 5 days previously. At the same time, the recipients were given EAE cells prepared in the conventional manner from donors that had not received any drugs. The added spleen cells did not affect the lesions or signs in either the localized or nonlocalized forms of EAE (3 and 5 recipients, respectively), compared to recipients given saline instead of spleen cells.

The possibility that tilorone induced suppressor cells in donor rats with EAE was approached with the aid of spleens from the immunized, tilorone-treated donor rats in the fifth experiment of Table 3. A suspension containing 4.4×10^8 of these spleen cells was injected intravenously into naive recipient rats and, 1 day later, these same recipients were given EAE cells from donors that had not received any drugs. Again, the added spleen cells did not affect the localized form of EAE compared to controls (groups of 4 rats). In nonlocalized EAE, 2/4 spleen cell-treated recipients developed clinical signs, compared to 6/6 controls. However, the histological scores were too close (average 2.5 vs. 3.0) to consider this significant. We conclude that no support for the suppressor cell hypothesis was obtained under the particular conditions of these experiments.

DISCUSSION

Production of EAE by passive transfer instead of active sensitization is advantageous for investigation of immunopharmacologic mechanisms because it permits some dissection of the immune response. The localized form of passive EAE has the additional advantages of greater speed and sensitivity and greater control of the age and type of the cellular infiltrates (reviewed by Levine, 1974). For the present investigation, the greatest advantage was the development of the immune response in one day, because the interferon response to tilorone also peaked in one day (Rhoads, 1973). Interferon is known to be immunosuppressive and to suppress EAE in rats (Abreu, 1982; Abreu et al., 1983). Nevertheless, the absence of detectable interferon, despite inhibition of EAE after treatment with tilorone under certain conditions of dose and timing, provided strong evidence against a role for induced interferon in the immunosuppressive activity of the drug. Absence of interferon in the serum after an EAE model of longer duration would be less significant evidence because interferon levels fall in a few days, even if tilorone treatments are repeated (tachyphylaxis) (Rhoads, 1973). Many of tilorone's other effects, even including instances of protection against virus infection, do not correlate with interferon induction (Levin

and Albrecht, 1981). Depletion of T lymphocytes is probably closer to the heart of tilorone's immunosuppressive activity than interferon induction, a concept supported by the ALS experiments. The relatively long duration of tilorone's inhibitory effect (Tables 1 and 3) correlates with the long persistence of tilorone in the tissues (Hook et al., 1974), even if it does not correlate with interferon levels.

The trials of tilorone-interferon combinations were based on the priming effect of small doses of interferon (Stewart et al., 1971). That is, cells in tissue cultures pretreated with small amounts of interferon will respond to viral induction with increased interferon production. No enhancement in the induction of interferon or in the suppression of EAE by tilorone was observed when the animals were pretreated with small, suboptimal doses of interferon. This failure to enhance interferon levels may be due to differences between in vivo and in vitro conditions and/or to differences between mechanisms of interferon induction by viruses and by tilorone.

Our data suggest that tilorone has multiple points of attack on the developmental pathway for production of EAE. The reduced yield and weak encephalitogenicity of donor cells after tilorone treatment on the day of immunization suggest that the drug interfered with the clonal expansion in response to antigen. The fact that EAE was reduced after these donor cells resided in the tilorone-free recipient for either 1 or 8 days indicates that the deficit was not remediable, at least not under these conditions where contact with antigen had been interrupted. Of course, these very same donors would have developed EAE a few days later if they had not been killed after 8 days, so the deficit was soon corrected when there was continued contact with antigen.

On the other hand, late tilorone treatment of donors (6 or 7 days after immunization) did not prevent progression of signs in these animals. This might be explained by the low tilorone content of blood and brain (Hook et al., 1974) which might have exempted EAE cells in transit, or already in the target neural tissues, from immunosuppression. The strong affinity of tilorone for lymphoid tissue might explain the suppressive effect of this late treatment on the encephalitogenicity of lymph node cell transfer in the localized 1-day system. The considerable recovery of these cells in the 4-day and nonlocalized 8-day systems can be attributed to dissipation of drug effects in the tilorone-free environment of the recipient and to the independence of the cells from continued antigenic stimulation once clonal expansion has been completed.

The effect of a drug on the nonspecific reactive cells that respond to injury created by the specific EAE cells can sometimes be elucidated by the passive transfer system. A rapidly dissipated drug like cyclophosphamide, injected 1 day before transfer of EAE cells, can alter or destroy nonspecific cells in the recipient without any risk of damaging donor cells (Levine, 1974). Tilorone's long persistence in tissues does not permit this kind of limited interaction, and the drug probably affected both donor and recipient cells no matter when it was given to recipients. However, the fact that tilorone was equally or more effective on the day before cell transfer as on the day of transfer (Table 1) suggests that at least part of its effect was on recipient (nonspecific) cells. If it suppressed only donor (specific) cells, a decrease in activity might have occurred due to metabolism and excretion during the day before EAE transfer. Kettman (1978) also found evidence of effects of tilorone on both specific and nonspecific cells in delayed hypersensitivity responses to sheep erythrocytes.

Supported by research grants from the National Multiple Sclerosis Society (SL) and The Kroc Foundation (SLA). Tilorone was kindly donated by Merrell-Dow Pharmaceuticals, Inc.

REFERENCES

Abreu SL (1982) Suppression of experimental allergic encephalomyelitis by interferon. *Immunol Comm* 11:1.

Abreu SL, Cannella MS, Hanke J (1981) Induction and cross-species activity of rat fibroblast-derived interferon. *Microbios* 30:181.

Abreu SL, Tondreau J, Levine S, Sowinski R (1983) Inhibition of passive localized experimental allergic encephalomyelitis by interferon. *Int Arch All Appl Immunol* in press.

Hook RH, Williams JM, Bruzie GF, Wright GJ (1974) Metabolic disposition of tilorone hydrochloride in mice, rats and dogs. *Toxicol Appl Pharmacol* 29:145.

Kettman JR (1978) Modulation of the acquisition and expression of immunity by tilorone: I. Delayed-type hypersensitivity responses. *Immunopharmacology* 1:21.

Levin RH, Albrecht WL (1981) Tilorone and related bis-basic substituted polycyclic aromatic and heteroaromatic compounds. *Progr Med Chem* 18:135.

Levine S (1974) Hyperacute, neutrophilic and localized forms of experimental allergic encephalomyelitis: A review. *Acta Neuropathol* 28:179.

Levine S (1977) T-cell depletion and other effects of tilorone. In *Modulation of host immune resistance in the prevention or treatment of induced neoplasia.* Ed. MA Chirigos. Washington, DC: Fogarty International Center Proceedings No. 28, U.S. Government Printing Office, p. 89.

Levine S, Gibson JP, Megel H (1974) Selective depletion of thymus dependent areas in lymphoid tissue by tilorone. *Proc Soc Exp Biol Med* 146:245.

Levine S, Hoenig EM (1968) Induced localization of allergic adrenalitis and encephalomyelitis at sites of thermal injury. *J Immunol* 100:1310.

Levine S, Sowinski R (1976) Necrotic myelopathy (myelomalacia) in rats with experimental allergic encephalomyelitis treated with tilorone. *Am J Pathol* 82:381.

Levine S, Sowinski R (1977) Synergistic suppression of the hyperacute form of experimental allergic encephalomyelitis by tilorone and cycloleucine. *Proc Soc Exp Biol Med* 156:457.

Levine S, Cacho P, Sowinski R, Thelmo WL (1983) Depletion of lymphoid tissues by tilorone: ultrastructure and synergistic interaction with antilymphocyte serum. *Am J Pathol* in press.

Levine S, Sowinski R, Hoenig EM (1975) Nuclear bodies produced in astrocytes by tilorone. *Am J Pathol* 78:319.

Lin L, Abreu SL (1979) Production, purification and characterization of rat interferon. *Arch Virol* 62:221.

Megel H, Raychaudhuri A, Goldstein S, Kinsolving CR, Shemano I, Michael JG (1974) Tilorone: its selective effects onhumoral and cell-mediated immunity. *Proc Soc Exp Biol Med* 145:513.

Megel H, Raychaudhuri A, Shemano I, Gibson JP (1977) Immunological responses with tilorone. In *Modulation of host immune resistance in the prevention and treatment of induced neoplasias.* Ed. MA Chirigos. Washington, DC, Fogarty International Center Proceedings No. 28, U.S. Government Printing Office, p. 103.

Rhoads AR, West WL, Morris HP (1973) Interferon induction and suppression of solid tumor growth in rats by tilorone hydrochloride. *Res Comm Chem Path Pharm* 6:741.

Stewart WE II, Gosser LB, Lockart RZ Jr (1971) Priming: a nonantiviral function of interferon. *J Virol* 7:792.

Stringfellow DA, Glasgow LA (1972) Tilorone hydrochloride: an oral interferon-inducing agent. *Antimicrob Ag Chemother* 2:73.