Cancer Immunol Immunother (1982) 14:41–45

Cancer Immunology Immunotherapy

Endotoxin-Induced Release of Tumour Necrosis Factor and Interferon in vivo is Inhibited by Prior Adrenoceptor Blockade

Nanne Bloksma, Frans Hofhuis, Barry Benaissa-Trouw, and Jan Willers

Department of Immunology, Laboratory of Microbiology, State University of Utrecht, Catharijnesingel 59, NL-3511 GG Utrecht, The Netherlands

Summary. *The effect of α- and β-adrenoceptor blocking agents on endotoxin-induced release of tumour necrosis factor (TNF), and of interferon in the circulation of Corynebacterium parvum-treated mice was the subject of this study. TNF was quantified after injection of TNF containing heated serum (TNS) into Meth A sarcoma-bearing mice by determining colour, extent, and incidence of haemorrhagic necrosis. The release of TNF was weakly inhibited by the competitive α-blocker phentolamine and the β-blocker propranolol. The non-competitive α-blocker phenoxybenzamine inhibited to a higher degree. Endotoxin-induced elicitation of growth-inhibiting principles into TNS was antagonized by propranolol and phenoxybenzamine. Administration of adrenaline before endotoxin inhibited the elicitation of TNF and growth-inhibitory activities, which indicates tachyphylaxis. The release of interferon was effectively inhibited by both α-adrenoceptor blockers but not by propranolol. The interferon was heat-labile. The results indicate that endotoxin-induced TNF and interferon are separate factors, elicited in different ways. As both α-blockers do not only inhibit reactions at the α-adrenergic receptor but also reactions at the serotonin receptor and in the case of phenoxybenzamine also at the choline receptor, it is suggested that endotoxin-induced release of the anti-tumour factors is controlled by reactions mediated by one or more of these receptors. It is suggested that the inhibition of TNF release by propranolol may be due to the membrane-stabilizing activity of this agent.*

Introduction

Among the many effects that can be observed after administration of endotoxin to an animal are the induction of haemorrhagic necrosis in subcutaneously transplanted tumours, with tumour regression in a fixed number of instances [1] and the appearance of interferon in the circulation [27].

Haemorrhagic necrosis is believed to be an indirect effect of endotoxin as this agent is not toxic for tumour cells in vitro [20, 25]. Moreover, haemorrhagic necrosis can be induced by a factor elicited by endotoxin in vivo, hence called tumour necrosis factor (TNF) [7]. TNF is a glycoprotein with a molecular weight of 70,000 daltons [15], and is probably produced by macrophages [7]. Optimal quantities of TNF were elicited in the serum of *Corynebacterium parvum*-treated mice 90 min after IV injection of endotoxin [9]. Almost identical treatment appeared to be optimal for the induction of interferon in serum [27]. Even injection of endotoxin into untreated mice, however, induced considerable quantities of interferon, while elicitation of measurable amounts of TNF required pretreatment with macrophage-activating agents such as *C. parvum,* bacille Calmette-Guérin, and zymosan [7]. The endotoxin-induced interferon was shown to be mainly of macrophage origin [10, 22] and could be readily inactivated by heating at 56° C [12, 26], while TNF was shown to be stable at this temperature. Serum with TNF (TNS) appeared to be cytostatic for Meth A sarcoma cells in vitro [4]. Recently we have demonstrated that the induction of haemorrhagic necrosis of Meth A sarcoma by endotoxin was inhibited by prior administration of the α-adrenoceptor blocking agents phentolamine and phenoxybenzamine. The latter drug also antagonized the growth retardation and regression of the tumours induced by endotoxin [5]. Histopathological examination showed that endotoxin induced a profound inhibition of the mitotic activity within Meth A tumours 4 and 24 h after its administration. The latter effect was not affected by prior administration of phenoxybenzamine [14].

In the present study the effect of adrenoceptor-blocking agents on the elicitation of TNF and interferon by endotoxin in vivo was investigated. In addition the role of adrenaline in endotoxin-induced TNF release was studied.

Materials and Methods

Mice. Female Swiss random mice were bought from the Central Institute for the Breeding of Laboratory Animals (CPB, Zeist, The Netherlands). Female BALB/c inbred mice were bred and maintained in our own facilities. All mice were used at the age of 12 weeks.

Production of TNS. Swiss random mice each received 1 mg *C. parvum* IV (Coparvax, Burroughs Wellcome Research Laboratories, Beckenham, Kent, England) and 25 μg endotoxin IV (LPSw from *E. coli* 0111 : B4, Difco Laboratories, Detroit, Michigan, USA) 14 days later. Blood was collected 90 min after endotoxin injection. The adrenoceptor-blocking agents phenoxybenzamine, (15 mg/kg body weight), kindly provided by Smith, Kline & French (The Hague, The Netherlands), phentolamine (50 mg/kg, Ciba-Geigy N.V., Groot Bijgaarden, Belgium), and propranolol (50 mg/kg, Sigma Chemical Company, St Louis, Missouri, USA) were diluted with or dissolved in saline and administered IP 15 min before endotoxin. The doses of α-adrenoceptor blocking agents used have been shown to inhibit endotoxin-induced haemorrhagic necrosis

Reprint requests should be addressed to N. Bloksma

0340-7004/82/0014/0041/$ 01.00

[5, 14]. Adrenaline (1 mg/kg, Sigma, St. Louis, USA) was dissolved in phosphate-buffered saline and a few drops of 0.1 *N* HCl. The pH was adjusted to 7.0 with 0.1 *N* NaOH. It was injected IV 30 min before endotoxin. The dose used was shown to induce haemorrhagic necrosis of murine sarcoma [2, 17; N. Bloksma et al., unpublished work].

Determination of TNF. TNF was quantified by determination of the colour, extent, and incidence of haemorrhagic necrosis after IV injection of 0.5 ml heated (10 min 56° C) serum into BALB/c mice bearing a subcutaneous Meth A sarcoma (mean diameter about 7 mm). The size of the tumour and the haemorrhagic necrotic area were measured with a caliper. The percentage of haemorrhagic necrosis was calculated by the formula:

$$\frac{\text{length + breadth of the necrotic area}}{\text{length + breadth of the tumour}} \times 100\% .$$

Quantitation of Interferon. Interferon was assayed by the plaque reduction method [23]. In brief, monolayers of mouse L cells (clone 929) were propagated in Eagle's minimum essential medium (MEM) and 10% heated (30 min at 56° C) calf serum (National Institute of Public Health, Bilthoven, The Netherlands) in 6 mm diameter wells (Titertek, Flow Laboratories, Great Britain) at 37° C. They were incubated with 0.1 ml volumes of twofold dilutions of the serum samples in MEM for 18–24 h, after which 30–40 plaque-forming units of vesicular stomatitis virus were adsorbed on each well for 1 h at 20° C. The wells were overlaid with 50 µl MEM containing 5% calf serum and 0.5% Oxoid agar no. 1 (Oxoid Ltd, Great Britain), and incubated for 18–24 h at 37° C. Plaques were visualized by flooding the agar overlay with 0.02% neutral red in Hank's balanced salt solution. One unit of interferon was defined as the dilution of serum causing 50% decrease in the plaque number. One unit of our interferon corresponded to 2.8 U of the mouse interferon standard of the National Institutes of Health (Bethesda, Maryland, USA). Data have been expressed as laboratory units.

Statistics. When appropriate data have been expressed as mean ± SEM. Significance analysis was done by Student's *t*-test or the Mann-Whitney U-test [21], as indicated in the legends. *P*-values over 0.05 were considered not significant.

Results

In Vivo Anti-Tumour Activity of TNS Prepared During Adrenoceptor Blockade

Injection of 0.5 ml normal TNS IV caused 100% incidence of dark-coloured haemorrhagic necrosis in tumour-bearing mice (Table 1). The necrosis measured on average 62.2% of the tumour size. Necrosis was followed by a growth stop in eight out of ten tumours, and in one mouse the tumour disappeared completely. The same amount of TNS which had been prepared in mice treated with one of the adrenoceptor antagonists before elicitation with endotoxin caused a lower incidence of necrosis. Adrenoceptor blockade with propranolol and especially phenoxybenzamine yielded sera which caused less dark-stained necrosis in addition. Only the latter serum caused a reduced extent of the necrotic area. Necrosis was followed less frequently by a stagnation of tumour growth after injection of 0.5 ml TNS from mice treated with phenoxybenzamine or propranolol prior to endotoxin, while the incidence of regression was the same as in the control group. TNS obtained after blockade with phentolamine induced a higher incidence of regression. Injection of 0.2 ml normal TNS resulted in reduced necrosis with respect to all parameters. An equal dose of TNS obtained from mice during blockade with phentolamine and propranolol had about the same anti-tumour activity as normal TNS, but TNS from mice treated with phenoxybenzamine was almost devoid of anti-tumour action.

Induction of Interferon by Endotoxin During Adrenoceptor Blockade

Endotoxin induced more interferon in *C. parvum*-treated mice than in normal untreated animals (Tables 2 and 3). When the α-adrenoceptor blocking agents phentolamine or phenoxybenzamine were administered before endotoxin less interferon

Table 1. In vivo anti-tumour activity of TNS elicited by endotoxin after adrenoceptor blockade of *C. parvum*-treated mice

Treatment of serum donors	Quantity of serum	Necrosis			Incidence of	
		Incidence[a]		Extent[b]	Growth stop[c]	Regression[d]
		Dark	Light			
Cp/saline/endotoxin[e] (TNS)	0.5 ml	10/10	0/10	62.2 ± 3.6	8/10	1/10
Cp/PA/endotoxin	0.5 ml	6/10	0/10	61.9 ± 3.0	6/10	3/10
Cp/PB/endotoxin	0.5 ml	3/10	4/10	51.6 ± 4.8[f]	2/10	1/10
Cp/PP/endotoxin	0.5 ml	5/10	2/10	57.1 ± 3.5	4/10	1/10
Saline (NMS)	0.5 ml	0/10	0/10		0/10	0/10
Cp/saline/endotoxin	0.2 ml	0/10	2/10	36.0 ± 5.2	4/10	1/10
Cp/PA/endotoxin	0.2 ml	0/10	3/10	29.8 ± 10.1	3/10	0/10
Cp/PB/endotoxin	0.2 ml	0/10	0/10		1/10	0/10
Cp/PP/endotoxin	0.2 ml	0/10	1/10	55.8	4/10	0/10
Saline	0.2 ml	0/10	0/10		1/10	0/10

[a] The incidence of dark brown to black necrosis and red to light brown necrosis have been scored separately
[b] Mean ± SEM of necrotic tumours 2 days after injection of serum
[c] Growth stop was scored as positive when tumour size did not increase for at least 2 days after serum injection
[d] Total disappearance of the tumour within 12 days after serum administration
[e] Abbreviations used: Cp, *C. parvum*; PA, phentolamine; PB, phenoxybenzamine; PP, propranolol
[f] $P < 0.05$, compared with TNS (*t*-test)

Table 2. Effect of adrenoceptor-blocking agents on serum interferon induced by endotoxin in normal and *C. parvum*-treated mice

Blocker/elicitor	Interferon (U/0.1 ml, median)[a]	
	C. parvum-pretreated	Normal
Saline/endotoxin	441	156[b]
Phentolamine/endotoxin	49[b]	42[d]
Phenoxybenzamine/endotoxin	174[c, e]	< 20[d]
Propranolol/endotoxin	617	158[e]
Saline/saline	< 20	< 20
Phentolamine/saline	nd[f]	< 20
Phenoxybenzamine/saline	nd	< 20
Propranolol/saline	nd	< 20

[a] Per group sera of four individual mice were tested in quadruplicate. For significancy analysis (U test) values < 20 U have been equalized to 20
[b] $P = 0.014$ compared with *C. parvum*/saline/endotoxin treatment (U-test)
[c] $P = 0.028$ compared with *C. parvum*/saline/endotoxin treatment (U-test)
[d] $P = 0.014$ compared to saline/endotoxin treatment (U-test)
[e] $n = 3$
[f] nd, not done

Table 3. Thermolability of endotoxin-induced serum interferon

Treatment of serum donors	Interferon (U/0.1 ml)[a]	
	Normal serum	Heated serum[b]
C. parvum/endotoxin	720	22[c]
−/endotoxin	284[c]	22[d]

[a] Median value of four determinations in pooled serum of four mice
[b] Heat treatment for 10 min at 56° C
[c] $P = 0.014$, compared with normal *C. parvum*/endotoxin serum (U-test)
[d] $P = 0.014$, compared with normal −/endotoxin serum (U-test)

could be measured in the serum (Table 2). β-Adrenoceptor blockade with propranolol did not affect interferon induction by endotoxin significantly. Administration of either *C. parvum* or blocking agent alone yielded no substantial quantities of interferon. The interferon induced by endotoxin in both *C. parvum*-treated and normal mice appeared to be heat-labile (Table 3).

Possible inhibitory activity on the interferon determination by residual blocking agents in the serum was tested by addition of serum of mice treated only with blocker to serum of endotoxin-treated mice. None of the sera of blocker-treated mice affected the measurement of interferon in post-endotoxin serum (Table 4).

Induction of Interferon by Endotoxin and TNS in Tumour-Bearing Mice

The anti-tumour activities of 25 μg endotoxin and 0.5 ml heated TNS in mice with Meth A sarcoma are comparable [7]. We measured the capacity of these agents to induce interferon in the circulation of mice with 9-day-old Meth A sarcoma. Blood was collected 90 min after IV injection of either agent. Only endotoxin induced substantial quantities of interferon (Table 5).

Table 4. Effect of residual blocking agents in the serum on interferon levels measured in post-endotoxin serum

Serum mixtures[a] of mice treated with	Interferon (U/0.1 ml)[b]
−/endotoxin and −/saline/saline (0)[c]	347
−/endotoxin and −/phenotolamine/saline (4)	309
−/endotoxin and −/phenoxybenzamine/saline (7)	284
−/endotoxin and −/propranolol/saline (0)	354

[a] Equal quantities of pooled sera were mixed prior to assay
[b] Median of four determinations
[c] Figures in parentheses indicate interferon titres of the sera added

Table 5. Induction of interferon by endotoxin and TNS in tumour-bearing mice[a]

Agent	Interferon (U/0.1 ml)[b]
Endotoxin	568
TNS	< 75[c]
−/endotoxin serum	< 75[c]
NMS	< 75[c]

[a] Groups of five BALB/c mice each bearing a 9-day-old Meth A sarcoma received 25 μg endotoxin or 0.5 ml heated serum IV. Blood was withdrawn after 90 min
[b] Median of five mice, each tested in quadruplicate
[c] $P = 0.014$ compared to endotoxin (U-test)

Table 6. Effect of adrenaline on the elicitation of TNF by endotoxin

Treatment of serum donor	Necrosis			Incidence of	
	Incidence[a]		Extent[b]	Growth stop[c]	Regression[d]
	Dark	Light			
Cp/saline/endotoxin (TNS)	4/5	0/5	66.2 ± 3.8	4/5	3/5
Cp/adrenaline/endotoxin	1/5	2/5	46.6 ± 2.2[e]	1/5	1/5
Cp/adrenaline	0/5	0/5		0/5	0/5
Saline (NMS)	0/5	0/5		0/5	0/5

[a] Dark and light brown necrosis habe been scored separately
[b] Mean ± SEM of necrotic tumours 2 days after injection of serum
[c] No increase of tumour size for at least 2 days after serum injection
[d] Total disappearance of the tumour within 12 days after serum injection
[e] $P < 0.05$ compared with TNS (*t*-test)

Effect of Adrenaline on the Elicitation of TNF by Endotoxin

The inhibitory effect of adrenoceptor-blocking agents on the endotoxin-induced TNF production may indicate that endotoxin somehow acts by release of adrenaline. Blood collected 90 min after injection of adrenaline into *C. parvum*-treated mice contained no demonstrable TNF or other tumour-inhibiting principles (Table 6). When adrenaline was administered before endotoxin the serum obtained caused less intensely coloured and significantly less extensive haemorrhagic necrosis, growth stop, and regression than normal TNS.

Discussion

The effect of adrenoceptor-blocking agents on the endotoxin-induced in vivo release of TNF and interferon was the subject of this study. A decrease of interferon was observed after treatment with the α-adrenoceptor blocking agents phentolamine and phenoxybenzamine (Table 2). Only the latter blocker clearly inhibited the release of TNF (Table 1). The decreased interferon titres in the serum of mice treated with α-antagonists are probably due to an inhibited production and/or release in vivo, as sera of mice treated only with blocker did not affect the measurement of interferon in vitro (Table 4). The observation that TNF production in *C. parvum*-treated mice is significantly inhibited by the non-competitive α-adrenoceptor blocker phenoxybenzamine [24], while the competitive α-blocker phentolamine had much less inhibitory activity, is in line with their capacity to inhibit the induction of haemorrhagic necrosis by endotoxin in tumour-bearing mice [5] and suggests that the latter might be at least partly due to reduced availability of TNF. It is questionable, however, whether the induction of haemorrhagic necrosis by endotoxin in tumour-bearing mice is mediated by TNF, as we could not demonstrate measurable quantities of TNF in the serum of endotoxin-treated tumour-bearing mice [2]. Moreover, the necrotizing principle could not be removed from TNS by in vitro absorption with the tumour target cells [2; N Bloksma et al., unpublished work]. These observations are consistent with data of Kull and Cuatrecasas [13], who could not detect cytotoxic activity against L cells in vitro in serum of tumour-bearing mice after injection of a necrotizing dose of endotoxin, while the purified necrotizing principle from TNS had overt cytotoxic properties in vitro.

Besides haemorrhagic necrosis TNS induced temporal stagnation of tumour growth and incidentally tumour regression (Table 1). The elicitation by endotoxin of growth-reducing mediators in TNS was considerably inhibited by propranolol, and especially by phenoxybenzamine. These results are only partly consistent with previous data [5]. Phenoxybenzamine, but not phentolamine, clearly antagonized the tumour growth-inhibiting effects of endotoxin in Meth A sarcoma-bearing mice, while propranolol rather potentiated the tumour growth inhibition induced by endotoxin. Propranolol possibly delays rather than prevents factor release, as a consequence of its membrane stabilizing activity [24]. This is likely as many endotoxin-induced factors are thought to be released by the membrane labilizing activity of endotoxin [8, 19]. On the other hand interferon release is not at all diminished by β-blockade (Table 3). Nevertheless, glucocorticosteroids, pre-eminently membrane-stabilizing agents, readily inhibited the induction of interferon by endotoxin in rabbits [16]. Apparently there is no direct relation, if any at all, between membrane stability and endotoxin-induced interferon release.

Inhibition of tumour growth after injection of endotoxin [5] or heated TNS (Tables 1 and 6) may be due to decreased mitotic activity within the tumour [2, 14]. Interferon and/or heat-stable cytostatic principles induced by endotoxin [3] may mediate this. A major role of interferon in the observed anti-tumour activities, however, is questionable. α-Adrenoceptor blockade with phenoxybenzamine appeared to inhibit the induction of interferon by endotoxin (Table 2) but did not influence the induction of reduced mitotic activity [14]. In addition, phentolamine hardly antagonized the induction of tumour growth inhibition by endotoxin [5], while it inhibited induction of interferon effectively (Table 2). Moreover, heated TNS induced no substantial quantities of interferon (Table 5), while its anti-tumour activity in vivo is about equal to that of endotoxin.

The inhibition of interferon production (Table 2) may be related to the observations of Jensen [11] that high doses of adrenaline given 1 h before endotoxin decreased the interferon release in the serum induced by the latter. Butler et al. [6] showed that the release of colony-stimulating factor in the serum of mice by endotoxin in vivo was inhibited by pretreatment with adrenaline. These data indicate tachyphylaxis and thus an adrenoceptor-mediated mechanism. Administration of adrenaline in a dose which was shown to be capable of inducing haemorrhagic necrosis (N. Bloksma et al., unpublished work) failed to elicit TNF or tumour growth-reducing principles in the circulation of *C. parvum*-treated mice. On the other hand, adrenaline given before endotoxin reduced the elicitation of both necrotizing and tumour growth-inhibiting activities by endotoxin (Table 6). These results, combined with the property of endotoxin of inducing the release of adrenaline [18] and the observation that adrenalectomized mice do not produce TNF (C. Galanos, personal communication cited in [9]), suggests that adrenaline has a kind of permissive action in the elicitation of TNF by endotoxin, which may be mediated by the α-adrenergic receptor. As phentolamine and phenoxybenzamine antagonize not only effects mediated by α-adrenergic receptors but also effects mediated by serotonin receptors, while the latter agent also antagonizes cholinergic effects [24], a role of serotonin and/or acetylcholine in the effects of endotoxin cannot be excluded.

Acknowledgements. Nanne Bloksma is supported by a grant from the Koningin Wilhelmina Fonds, Netherlands Cancer Foundation.

References

1. Berendt MJ, North RJ, Kirstein DP (1978) The immunological basis of endotoxin-induced tumor regression. Requirement for T-cell-mediated immunity. J Exp Med 148: 1550
2. Bloksma N (1982) Anti-tumour activities of endotoxin in the mouse. A focus on the role of tumour necrosis factor. PhD thesis, State University of Utrecht, The Netherlands
3. Bloksma N, Hofhuis FMA, Schetters TP, Willers JMN (1980a) In vitro antitumour activity of tumour necrosis serum, role of natural antibodies and complement. In: Israël L, Lagrange P, Salomon JC (eds) Cancer immunology and parasite immunology. INSERM 97: 223
4. Bloksma N, Schetters TP, Figdor C, Van Dijk H, Willers JM (1980b) In vitro anti-tumour activity of tumour necrosis serum. Int J Immunopharmacol 2: 95
5. Bloksma N, Hofhuis FM, Willers JMN (1982) Effect of adrenoceptor blockade on hemorrhagic necrosis of Meth A sarcomata induced by endotoxin or tumour necrosis serum. Immunopharmacology 4: 163
6. Butler RC, Abdelnoor AM, Nowotny A (1978) Bone marrow colony-stimulating factor and tumour resistance-enhancing activity of post-endotoxin mouse sera. Proc Natl Acad Sci USA 75: 2893
7. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B (1975) An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci USA 72: 3666
8. Cohn ZA, Morse SI (1960) Functional and metabolic properties of polymorphonuclear leucocytes. II. The influence of a lipopolysaccharide endotoxin. J Exp Med 111: 689
9. Green S, Dobrjansky A, Chiasson MA, Carswell E, Schwartz MK, Old LJ (1977) *Corynebacterium parvum* as the priming agent in the production of tumor necrosis factor in the mouse. J Natl Cancer Inst 59: 1519

10. Ho M, Breinig MC, Maehara N (1976) Cellular basis of interferon formation and hyporeactivity after exposure to bacterial lipopolysaccharide. J Infect Dis 133: [suppl] A30
11. Jensen MM (1969) The influence of vasoactive amines on interferon production in mice. Proc Soc Exp Biol Med 130: 34
12. Kato N, Nakashima L, Ohta M, Naito S, Kojima T (1979) Interferon and cytotoxic factor (cytotoxin) released in the blood of mice infected with *Mycobacterium bovis* BCG. I. Enhanced production of interferon and appearance of cytotoxin stimulated by capsular polysaccharide of *Klebsiella pneumoniae* or bacterial lipopolysaccharide. Microbiol Immunol 23: 383
13. Kull FC, Cuatrecasas P (1981) Preliminary characterization of the tumor cell cytotoxin in tumor necrosis serum. J Immunol 126: 1279
14. Kuper CF, Bloksma N, Hofhuis FM, Bruyntjes JP, Willers JM (1982) Influence of adrenoceptor blockade on endotoxin-induced histopathological changes in murine Meth A sarcoma. Int J Immunopharmacol 4: 49
15. Oettgen HF, Carswell EA, Kassel RL, Fiore N, Williamson B, Hoffmann MK, Haranaka K, Old LJ (1980) Endotoxin-induced tumor necrosis factor. In: Mathé G, Muggia FM (eds) Cancer chemo- and immunopharmacology. 2. Immunopharmacology, relations and general problems. Springer-Verlag, Berlin Heidelberg New York, p 207
16. Postic B, DeAngelis C, Breinig MK, Ho M (1967) Effects of cortisol and adrenalectomy on induction of interferon by endotoxin. Proc Soc Exp Biol Med 125: 89
17. Pradhan SN, Achinstein B, Shear MJ (1957) Independence of drug-induced damage in sarcoma 37 and carotic blood pressure in mice. Arch Exp Pathol Pharmakol 230: 228
18. Rosenberg JC, Lillehei RC, Moran WH, Zimmermann B (1959) Effect of endotoxin on plasma catechol amines and serum serotonin. Proc Soc Exp Biol Med 102: 335
19. Shands JW, Peavy DL, Gormus BJ, McGraw J (1974) In vitro and in vivo effects of endotoxin on mouse peritoneal cells. Infect Immun 9: 106
20. Shapiro CJ (1940) The effect of a toxic carbohydrate complex from *S. enteritidis* on transplantable rat tumors in tissue culture. Am J Hyg [B] 31: 114
21. Siegel S (1959) The case of two independent samples. In: Harlow HF (ed) Nonparametric statistics for the behavioural sciences. McGraw-Hill Kogakusha, Tokyo, p 95
22. Smith TJ, Wagner RR (1967) Rabbit macrophage interferons. I. Conditions for biosynthesis by virus-infected and uninfected cells. J Exp Med 125: 559
23. Wagner RR (1961) Biological studies of interferon. I. Suppression of cellular infection with Eastern equine encephalomyelitis virus. Virology 13: 323
24. Weiner N (1980) Drugs that inhibit adrenergic nerves and block adrenergic receptors. In: Goodman Gilman A, Goodman LS, Gilman A (eds) The pharmacological basis of therapeutics. Macmillan, New York, p 176
25. Yang C, Nowotny A (1974) Effect of endotoxin on tumor resistance in mice. Infect Immun 9: 95
26. Youngner JS, Salvin SB (1973) Production and properties of migration inhibitory factor and interferon in the circulation of mice with delayed hypersensitivity. J Immunol 111: 1914
27. Youngner JS, Stinebring W (1965) Interferon appearance stimulated by endotoxin, bacteria or viruses in mice pre-treated with *Escherichia coli* endotoxin or infected with *Mycobacterium tuberculosis*. Nature 208: 456

Received April 26/Accepted July 6, 1982