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The Inhibition of Endotoxin-Induced Local Inflammation by LDH Virus or LDH Virus-Infected Tumors Is Mediated by Interferon (42509)

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Abstract. The footpad swelling reaction induced by local injection of *S. marcescens* lipopolysaccharide was found to be inhibited in mice given a transplantable tumor (TA_3) or cell-free ascitic fluid from tumor-bearing mice. The tumor was shown to contain LDH virus, which is known to cause inapparent persistent infections in mice. Monoclonal antibodies directed against protein VP3 of the LDH virus could partially abrogate the anti-inflammatory effect of the TA_3-ascitic fluid, and, conversely, the anti-inflammatory effect could be obtained by LDH virus isolated from the tumor and reproduced by serial passage of cell-free fluids. Inhibition of the footpad reaction was seen in the acute but not in the chronic phase of LDH virus infection, suggesting that the anti-inflammatory effect might be due to endogenous interferon (IFN) which, similarly, was only detectable in the acute phase. Newcastle disease virus, another potent interferon inducer, had a similar inhibitory effect on the footpad reactivity. Moreover, the inhibitory effect of LDH virus infection could partially be abrogated by administration of a polyclonal antibody directed against murine IFN-α,β. Finally, passively administered natural murine IFN-α,β or recombinant murine IFN-α_1 (but not recombinant murine IFN-β) was found to cause inhibition of the footpad reaction. Since Gram-negative bacteria and their lipopolysaccharides have the ability to induce a systemic interferon response, our findings suggest that this interferon may play a modulatory role in local inflammation caused by these bacteria. Our findings also open a new perspective for interferon therapy of certain inflammatory reactions to bacterial infections. © 1987 Society for Experimental Biology and Medicine.

LDH virus is a togavirus which causes an inapparent and persistent infection in mice (1). Already in 1969 Howard *et al.* (2) noticed that the early stages of infection are associated with a state of immunosuppression, as evidenced by prolonged allograft survival. The virus is often found as a contaminant of transplanted murine tumors (1) and this has in several instances been found to account for immunosuppressive or anti-inflammatory effects of these tumors or their extracts (3, 4). To this date, the mechanism by which LDH virus infection exerts these effects has not been investigated. The virus infects only monocytes and macrophages (1). Since these cells play an important role in inflammation, it is conceivable that infection with LDH virus impairs their reactivity to inflammatory stimuli. Alternatively, the infection may cause production, release, or activation of host factors which exert immunosuppressive or anti-inflammatory effects. One possible candidate host factor is interferon. It is known indeed that interferon is produced during the early stages of LDH virus infection (5, 6) and that it has the ability to modulate activities of various cells that are involved in inflammation.

The inflammation model used for the present study consisted of footpad swelling in mice after local injection of bacterial (*Serratia marcescens*) lipopolysaccharide (LPS). While this reaction shares some characteristics with specific delayed-type hypersensitivity responses, previously shown to be inhibited by LDH virus (7) and interferon (8, 9), it also has properties of its own, in particular a thromboembolic component that in its most pronounced form is known as the Shwartzman phenomenon.

We here demonstrate that the footpad reaction to LPS is inhibited by LDH virus and LDH virus-infected tumors, and that this inhibition is for a large part, if not entirely, due to interferon induced by the virus.

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Materials and Methods. *Mice.* Female NMRI mice, 6 weeks of age, were obtained from the Experimental Animal Centre of the University of Leuven.

Viruses. LDH virus was an isolate from a transplantable TA_3 tumor (10). The strain of virus differed slightly from the standard Riley strain as indicated by a different migration pattern of the viral envelop proteins (see Results). Virus stocks were obtained as plasma or clarified (3000 rpm for 20 min at 4°C) ascitic fluid of tumor-bearing mice, and contained approximately $10^{6.4}$ to $10^{8.3}$ infectious doses (ID_{50})/ml. Virus titers were determined by inoculating 10-fold dilutions of serum or ascitic fluid (10 μl) intraperitoneally into groups of six mice, and screening the mice 5 days postinfection for the presence of plasma LDH activity (1). Virus stocks were stored at −70°C.

Newcastle disease virus (NDV, Komarov strain) was propagated on 10-day-old embryonated chicken eggs by intraallantoic inoculation and titrated by plaque assay on Vero cells.

Endotoxin. Lipopolysaccharide B (LPS) of *Serratia marcescens* was obtained from Difco (Detroit, MI.).

Interferon preparations. Mouse interferon α,β (MuIFN-α,β) was prepared on L929 cells induced with NDV (Komarov strain), concentrated and partially purified by fractional precipitation with ammonium sulfate (11). This interferon preparation had a specific activity of approximately $10^{5.7}$ units/mg protein.

Recombinant murine interferon-α_1 (rMuIFN-α_1) was derived from CHO-pSV10EF-3 cells (12) and kindly provided by Dr. J. Trapman (Erasmus University, Rotterdam, The Netherlands). The crude preparation had a titer of $10^{5.0}$ units/ml and a specific activity of approximately $10^{2.3}$ units/mg protein.

Recombinant murine interferon-β (rMuIFNβ) was derived from CHO-βAA_3 cells (13), also kindly provided by Dr. J. Trapman. The interferon was purified by adsorption to controlled pore glass as described for human IFN-β (14) giving a preparation with a specific activity of $10^{4.5}$ units/mg protein. A second batch was purified by affinity chromatography on a monoclonal antibody against MuIFN-β (15). The peak fraction had a specific activity of more than $10^{8.2}$ units/mg protein.

Blood samples for interferon determination on serum were taken from the orbital sinus. Infectious virus was inactivated by treatment at pH 2.0 before interferon assay. Interferon was titrated on primary mouse fibroblasts (MEF) or L929 cells using a CPE inhibition assay with mengovirus as a challenge (11). Unless indicated otherwise, interferon titers were expressed in units per milliliter in terms of the NIH standard preparation G002-904-511.

Antisera. Two monoclonal mouse anti-LDH virus antibodies were used in this study: AV24 containing five different monoclonal IgG2a antibodies and C3904H12, a monoclonal IgG3 antibody (16). Both antibodies are directed against VP_3, the glycosylated envelope protein of the virus. After addition of 50 μg of these antibodies to 30 μl of a preparation containing 3×10^9 ID_{50} of purified virus (incubation 30 min at 37°C and 2 hr at 4°C), the infectious titer was reduced by factors of 100 in the case of IgG2a and >1000 in the case of IgG3.

An antiserum against mouse IFN-α,β was obtained by immunizing a goat with NDV-induced L929 cell interferon (I. A. Braude, Ph.D. thesis, K. U. Leuven, 1978). The serum was absorbed on a suspension of mouse spleen and thymic cells and on L929 cells. The Ig fraction was precipitated by Na_2SO_4. The serum had a neutralization titer of 1/80,000 against 15 units/ml of MuIFN-α,β.

SDS-polyacrylamide gel. LDH virus was purified from plasma of acutely infected mice or from a tumor-bearing mouse by velocity centrifugation in 5 to 20% sucrose gradients at 35000 rpm for 105 min in a SW 40 rotor (Beckman Instruments, Inc., Palo Alto, CA). The virus was analyzed in a 10 to 15% gradient SDS-PAGE according to Laemmli (17), after staining with silver nitrate, following the method described by Morrissey (18).

Footpad assay. Mice were injected into the right hind footpad with 5 μg of LPS (in 25 μl). Phosphate-buffered saline (PBS), pH 7.4, was injected as a control into the left hind footpad. After 90 min, virus or other test materials were injected intraperitoneally (ip) or locally into the right footpad as stated in the text. Footpad swelling was measured daily for 6 days with the aid of micrometer calipers. Footpad swelling was calculated for individual mice as per-

centage increase in footpad thickness, by comparing the thickness of the LPS-injected footpad (L) with that of the contralateral injected footpad (C), using the formula

% increase in footpad thickness

$$= \frac{L - C}{C} \times 100. \quad [1]$$

A minimum of four mice were used for each group of control or treated mice. Percentage inhibition of footpad swelling in treated mice was calculated by the formula

% inhibition of footpad swelling

$$= 1 - \frac{\text{mean swelling in treated group}}{\text{mean swelling in control group}} \times 100. \quad [2]$$

For the control group of each experiment the 95% confidence limits for mean percentage increase in footpad thickness were calculated (Student's t test). Substituting the lower confidence limit for "mean swelling in treated group" in Formula [2], the corresponding confidence limit for "% inhibition of footpad swelling" was determined. The latter value is given in the footnotes to tables and figures as an index of precision of the assay.

Results. *Footpad reaction to LPS and its inhibition in mice inoculated with TA3 tumors.* Figure 1 shows the results of an experiment which illustrates the inhibition of LPS-induced footpad inflammation in mice carrying a transplantable TA3 tumor. Sets of four mice received injections of LPS in the right footpad. Immediately thereafter the mice were inoculated intraperitoneally with 2×10^7 TA3 tumor cells. Ascites, developing in the tumor-inoculated mice, became palpable on Day 3. Footpad swelling was monitored over a period of 6 days. Control mice showed increased footpad thickness with a maximal response (135%) at Day 2. Mice inoculated with TA3 cells showed a swelling up to 24 hr after LPS injection, which then regressed. At the time of peak reaction in the control mice, the tumor-inoculated mice had a swelling that averaged only 35%. Percentage inhibition on Day 2, as calculated by the formula given under Materials and Methods, amounted to 75% on Day 2 and 84% on Day 3. In two similar experiments maximal inhibition of footpad swelling in tumor-inoculated mice was 75 and 45%.

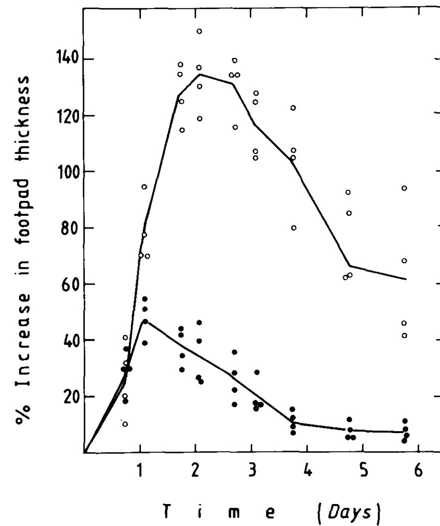

FIG. 1. Kinetics of LPS-induced footpad swelling in NMRI mice. (○) Control mice; (●) mice injected intraperitoneally with 2×10^7 TA3 tumor cells.

In order to determine whether suppression of the footpad swelling was due to a cell-free factor, a similar experiment was done using clarified TA3 ascitic fluid instead of TA3 cells. The injections were given subcutaneously (0.5 ml) or intraperitoneally (0.5 ml) daily from Day 0 to Day 5 post LPS challenge or only once subcutaneously, intraperitoneally, or locally in the right footpad (25 μl). The results illustrated in Fig. 2 show that significant suppression of the footpad reaction occurred in all cases. Even a single injection of undiluted ascitic fluid was sufficient to suppress the footpad swelling reaction.

To determine the potency of the anti-inflammatory activity of the TA3 ascitic fluids, 10-fold dilutions of three batches were injected 90 min after LPS challenge in the right footpad; 50% maximal inhibitory activities were found at dilutions ranging from $10^{-5.2}$ to $10^{-6.5}$.

This high level of biological activity of the TA3 ascitic fluid raised the possibility that it was due to a replicating agent present in the tumor and carried with it during transplantation. Since many transplantable mouse tumors are known to be contaminated with LDH virus (1), studies were undertaken to

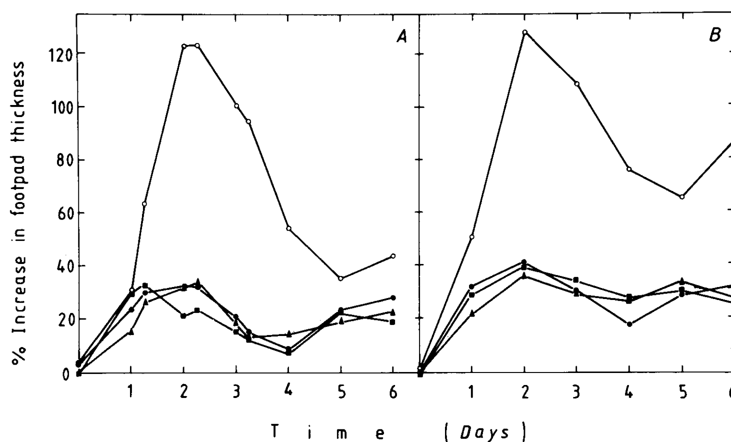

FIG. 2. Anti-inflammatory effect of cell-free tumor ascitic fluid on LPS-induced footpad swelling. Open symbols (○): controls receiving only LPS challenge in the footpad. Closed symbols: treated mice receiving TA3 ascitic fluid after LPS challenge: (●) 0.5 ml sc; (■) 0.5 ml ip; (▲) 25 μl locally in the footpad. (A) Multiple injections of TA3 fluid on Days 0, 1, 2, 3, 4, and 5. Statistical significance of differences between experimental groups and control: Day 2, $P < 0.02$; Day 3, $P < 0.05$; averages of four mice. (B) Single injections of TA3 fluid on Day 0, 90 min after LPS challenge. Statistical significance of differences between experimental groups and control: Day 2, $P < 0.001$; Day 3, $P < 0.02$; averages of five mice.

demonstrate that the anti-inflammatory effect of TA3 ascitic fluid was due to LDH virus.

Presence of LDH virus in transplanted TA3 tumors and its role in the inhibition of the footpad reaction. Transmissibility studies revealed that, after injection of TA3 ascitic fluid into normal mice, the serum of these mice could induce an inhibition of the footpad reaction. Inhibition was also detectable after serial mouse-to-mouse transfers of sera. Moreover, plasmas of mice that had received TA3 cells or ascitic fluid contained elevated levels of lactic dehydrogenase comparable to levels commonly observed in mice infected with LDH virus (3300–5000 units/liter). Finally, virus particles, possessing an SDS-PAGE profile closely resembling that of the Riley LDH virus strain, could be isolated from the plasma of a tumor-bearing mouse (Fig. 3). These observations led us to postulate that the anti-inflammatory effects of the TA3 tumor and ascitic fluid were due to the action of the contaminating LDH virus. Several experiments were done to test this hypothesis. Table I shows the results of an experiment in which we tested whether anti-LDH virus immunoglobulin could partially or completely abrogate the anti-inflammatory effect of the TA3 ascitic fluid. Two monoclonal antibodies directed against the VP3 protein of the LDH virus, which partially neutralized LDH virus infectivity, were used. Mixtures were prepared consisting of equal volumes of antibody, PBS, and diluted ascitic fluid, as indicated in Table I. The dilution of ascitic fluid was chosen so that the mice would receive 100 times the dose necessary to cause 50% maximal inhibition of the footpad reaction. The mixtures were incubated for 30 min at 37°C and 2 hr at +4°C and injected in the right footpad of groups of four mice 90 min after LPS challenge. The results in mice receiving both antibody and TA3 fluid were expressed as percentage inhibition in comparison with mice receiving neither TA3 fluid nor antibody or in comparison with mice receiving only the antibody. The C3904H12 monoclonal antibody by itself did not significantly affect the footpad reaction and clearly abrogated the inhibitory ability of TA3 ascitic fluid. The AV24 monoclonal antibody caused a slight footpad swelling by itself and was also less effective in suppressing the footpad inhibitory effect of the ascitic fluid. This was not unexpected since the AV24 antibody was known to possess a lower infectious neutralizing capacity than the C3904H12 antibody (16).

Since infection with LDH virus is followed by a lifelong persistent viremia (1), it was interesting to know whether inflammation in-

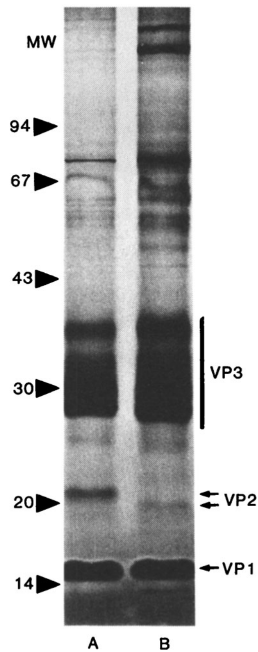

FIG. 3. SDS–polyacrylamide gel electrophoretic pattern of proteins associated with (A) LDH virus, Riley strain, obtained from the American Type Culture Collection (Rockville, MD) and (B) virus particles isolated from plasma of a TA3 tumor-bearing mouse.

TABLE I. EFFECT OF MONOCLONAL ANTIBODIES TO LDH VIRUS ON THE ANTI-INFLAMMATORY EFFECT OF TA3 TUMOR ASCITIC FLUID, AS TESTED BY THE LPS-INDUCED FOOTPAD SWELLING TEST

	Mixture injected[a]		% Inhibition of footpad swelling[b] on	
Groups	TA3 ascitic fluid	Anti-LDH virus globulin	Day 2	Day 3
1	−	−	0	0
2	+	−	53 (53)	58 (58)
3	−	+ (AV24)	−13 (0)	−22 (0)
4	+	+ (AV24)	29 (37)	32 (44)
5	−	+ (C3904H12)	4 (0)	5 (0)
6	+	+ (C3904H12)	14 (10)	5 (0.6)

[a] Ascitic fluid (diluted 10^{-3}) + antibody (undiluted) or PBS, v/v, incubated 30 min at 37°C and 2 hr at 4°C, was injected in the right footpad (25 μl), 90 min after LPS challenge.

[b] Calculated vs control mice (group 1): in parentheses, calculated vs appropriate control, i.e., group 2 vs group 1, group 4 vs group 3, and group 6 vs group 5; four mice per group; 95% confidence limit for % inhibition of footpad swelling: Day 2, 9%; Day 3, 22% (see Materials and Methods).

duced by LPS was still possible in chronically infected mice. For this purpose groups of four mice were injected intraperitoneally with TA3 ascitic fluid containing $10^{6.7}$ ID_{50} of LDH virus or with PBS. After 5 weeks, the mice were challenged in the right footpad with LPS and inoculated again in the same footpad with either $10^{5.1}$ ID_{50} LDH virus or control fluid. Table II indicates that mice chronically infected with LDH virus have footpad reactions comparable to those of control mice. Furthermore, reinfection with LDH virus was not able to suppress footpad reactivity to LPS in these mice.

Role of interferon in the anti-inflammatory effect of LDH virus infection. Several types of experiments were done to test the hypothesis that inhibition of the footpad reaction to LPS in the acute phase of LDH virus infection was due to systemic interferon. Specifically, we tested (i) whether LDH virus infection did induce interferon in our strains of mice; (ii) whether infection with another virus, NDV, known to be a good inducer of interferon, also caused inhibition of the LPS-footpad reaction; (iii) whether passively administered interferon inhibited the footpad reaction, and (iv)

TABLE II. LPS-INDUCED INFLAMMATION IN MICE CHRONICALLY INFECTED WITH LDH VIRUS

Group	Status of mice	Treatment given after LPS challenge	% Increase in footpad thickness[a] on Day 2	Day 3
1	Not infected	PBS	127	114
2	Mock infected[b]	LDH virus[c]	32	20
3		PBS	130	114
4	Chronically infected[d]	LDH virus	108	95
5		PBS	119	109

[a] Each value represents the mean of four mice; 95% confidence limits for % increase in footpad thickness in group 1: Day 2, ±28.2%; day 3, ±36.6% (see Materials and Methods).

[b] Mice were injected intraperitoneally 5 weeks before LPS challenge with 1 ml of PBS.

[c] $10^{5.1}$ ID_{50} in the right footpad 90 min after LPS.

[d] Mice were infected intraperitoneally 5 weeks before LPS challenge with 1 ml ascitic fluid containing $10^{6.7}$ ID_{50}/ml of LDH virus.

TABLE III. INTERFERON PRODUCTION IN SERA OF NMRI MICE INJECTED WITH LDH VIRUS OR NDV[a]

Inducer virus	Dose	Bleeding time (hr p.i.)	Interferon response: Number positive/total	Interferon response: Average titer of responders ($\log_{10}$ RU/ml)[b]
LDH virus	$10^{6.4}$ ID_{50}	0	0/10	<0.7
		24	7/7	2.4
		48	7/10	1.4
		72	0/10	<0.7
NDV	$10^{7.3}$ PFU	1	1/2	1.9
		3	2/2	4.15
		4	2/2	4.9
		6	2/2	>4.9
		24	2/2	4.15
NDV	$10^{6.6}$ PFU	1	1/2	1.9
		3	2/2	3.65
		4	2/2	4.0
		6	2/2	>4.9
		24	2/2	3.5

[a] LDH virus (cell-free TA3 ascitic fluid) was given intraperitoneally; NDV was given intravenously.
[b] Titration on MEF for LDH virus; on L929 cells for NDV.

whether inhibition of the footpad reaction by LDH virus could be abrogated by administration of anti-interferon immunoglobulin.

Table III shows the results of experiments done to measure production of circulating interferon in mice infected with LDH virus and NDV. Groups of mice were infected intraperitoneally with $10^{6.4}$ ID_{50} of LDH virus or intravenously with $10^{6.6}$ and $10^{7.3}$ PFU of NDV. At various time intervals the animals were bled and the sera were assayed for antiviral activity. The interferon-inducing capacity of LDH virus was modest in comparison with that of NDV. At 24 hr postinfection all tested mice had circulating interferon with a titer averaging $10^{2.4}$ units/ml. Prevalence and titers of circulating interferon were lower at 48 hr and became zero at 72 hr. In mice infected with NDV circulating interferon appeared within 1 hr postinfection and reached a maximal level in about 6 hr.

Table IV shows the results of experiments in which the ability of NDV infection to reduce footpad swelling reaction was examined. Groups of four NMRI mice were inoculated intravenously with 10^7 PFU of NDV at different time intervals starting from 5 hr before to 42 hr after LPS challenge. Footpad swelling was followed daily. Injection of NDV from 5 hr before to 18 hr after the LPS challenge invariably caused a 73 to 89% reduction in footpad swelling. Injection at 24 hr postchallenge was marginally effective; injection at 42 hr was ineffective.

In order to examine the effect of different types of interferon on the footpad reaction, groups of four mice were given intraperitoneal injections of natural MuIFN-α,β, recombinant MuIFN-α_1, or recombinant MuIFN-β. The

TABLE IV. EFFECT OF NDV ON THE LPS-INDUCED FOOTPAD SWELLING IN NMRI MICE

Time of NDV inoculation (hr)[a]	% Inhibition of footpad swelling[b] on Day 2	Day 3
−5	84.5	82
+1	89	80
+5	82	80
+18	80	73
+24	56	71
+42	−10	25

[a] 10^7 PFU of NDV were inoculated intravenously on indicated times before (−) or after (+) LPS challenge.
[b] Averages determined on four mice: 95% confidence limit for % inhibition of footpad swelling: Day 2, 19%; Day 3, 21% (see Materials and Methods).

TABLE V. Inhibition of LPS-Induced Footpad Swelling by Interferons: MuIFN-α,β, rMuIFN-α_1, and rMuIFN-β

Interferon type[a]	Dose ($\log_{10}$ units/mouse)	% Inhibition of footpad swelling[b] on	
		Day 2	Day 3
Experiment 1			
α/β (nat)	5.5	38	48
	4.5	35.5	52.5
	3.5	−4	0
Experiment 2			
α/β (nat)	4.8	52	62
α_1 (rec.)	5.0	53.5	73
β (rec.)[c]	4.0	1	4
Experiment 3			
α/β (nat)	5.0	32	41
α_1 (rec.)	5.0	43	55.5
	4.0	5	2
β (rec.)[d]	5.0	16	22
	4.0	−6	−22

[a] Interferon was given intraperitoneally each day from Day 0 to Day 3 post LPS challenge.
[b] Each value is an average determined on four mice: 95% confidence limit for % inhibition of footpad swelling: Day 2, 19%; Day 3, 27% (Experiment 1); Day 2, 11%; Day 3, 21% (Experiment 2); Day 2, 21%; Day 3, 86% (Experiment 3).
[c] Purified by affinity chromatography on monoclonal anti-IFN-β antibodies.
[d] Purified on CPG.

treatment was started on the day of LPS challenge in the footpad and a total of four injections were given on 4 consecutive days. Results are shown in Table V. It can be seen that high doses ($10^{4.3}$ to $10^{5.0}$ reference units) of MuIFN-α,β significantly depressed the footpad inflammatory reaction induced by LPS. The effect was dose dependent. rMuIFN-α_1 was as effective in depressing the footpad reaction as was natural MuIFN-α,β. In contrast, rMuIFN-β given at the same dose (10^5 units) caused only marginal inhibition of the footpad reaction. Unfortunately, insufficient amounts of pure rMuIFN-β were available to test the effect of higher doses.

If inhibition of footpad swelling in LDH virus-infected mice is mediated by interferon, it should be possible to abrogate this effect by giving antibodies which neutralize the biological activities of interferon. Groups of four mice were challenged by LPS in the footpad (Day 0). Intraperitoneal injections of antibody directed against natural MuIFN-α,β were given on Days −1, 0, 1, and 2. LDH virus ($10^{4.1}$ ID_{50}) or natural MuIFN-α,β ($10^{5.3}$ units) was given intraperitoneally on Day 0 (immediately after LPS), as well as on days 1, 2, 3, and 4. Table VI shows that LDH virus or MuIFN-α,β given alone caused a decrease in footpad swelling. In mice also receiving antibodies to MuIFN-α,β, the inhibition was less pronounced.

Discussion. Several authors have observed that transplanted tumors or certain tumor extracts can inhibit antigen-specific delayed-type hypersensitivity reactions or aspecific inflammatory responses (19–25) (e.g., skin reactivity toward plant lectins, bacterial lipopolysaccharides). Similarly, transfer of serum or ascites

TABLE VI. Effect of Antibodies to MuIFN-α,β on the Anti-inflammatory Effect of LDH Virus and MuIFN-α,β as Tested by the LPS-Induced Footpad Swelling Test

Group	Daily injection schedule[a]		% Inhibition of footpad swelling[b] on	
	Days −1 to 2	Days 0 to 4	Day 2	Day 3
1	PBS	LDH virus[d]	64	65
2	Anti-MuIFN-α,β[c]	LDH virus	39	40
3	PBS	MuIFN-α,β[e]	48	59
4	Anti-MuIFN-α,β	MuIFN-α,β	14	26
5	Anti-MuIFN-α,β		−4	0

[a] Day 0 = time of LPS challenge in footpad.
[b] Averages of four mice; 95% confidence limit for % inhibition of footpad swelling: Day 2, 10%; Day 3, 6%.
[c] 0.1 ml, neutralizing titer 1/80,000, ip.
[d] $10^{4.1}$ ID_{50}, ip.
[e] $10^{5.3}$ IU, ip.

of tumor-bearing mice has been reported to block or inhibit macrophage activity (21–23, 26) (e.g., chemotaxis, phagocytosis, attachment and spreading, tumoricidal activity). In many cases, there is evidence or suggestion that some of these effects are due to LDH virus present in the serum, ascites, or tumor extracts used. The experiments described here show that acute infection of mice with LDH virus results in a transient failure to mount a footpad swelling after local injection of *S. marcescens* lipopolysaccharide and that this inhibition is largely due to the interferon induced by the virus.

In our study inhibition of the footpad reaction by LDH virus was originally observed in experiments which involved inoculating the mice with the TA3 tumor. We could show that the tumor was carrying a strain of LDH virus and that the inhibition of the footpad reactivity by TA3 ascitic fluid could partially be blocked by prior incubation of the fluid with a monoclonal anti-LDH virus antibody. It is known that LDH virus causes a persistent infection of mice that is not accompanied by gross behavioral or pathological signs or symptoms (1). A viremic peak of 10^{10} to 10^{11} ID_{50}/ml occurs within 12–24 hr postinfection and is followed by lifelong persistent viremia (10^4 to 10^5 ID_{50}/ml). Circulating interferon is detectable only during the acute phase (Days 1 and 2) and correlates with the peak of circulating virus. Our evidence that the inhibition of the LPS-footpad reactivity is mediated by interferon, and not by persistence of the virus as such, is as follows:

(i) Circulating interferon and inhibition of footpad responsiveness, coincided temporally, both responses being restricted to the acute phase of the virus infection.

(ii) Inhibition of the footpad response could also be obtained by acute infection with an interferon-inducing dose of NDV, a virus which does not persist, but does induce a short-lived circulating interferon response.

(iii) Circulating interferon induced by the virus is of the α/β type, and inhibition of the footpad response by LDH virus infection could partially be abrogated by administration of a polyclonal rabbit anti-mouse IFN-α,β antibody preparation.

(iv) Passive administration of a natural mixture of MuIFN-α,β or recombinant DNA-derived MuIFN-α_1 caused inhibition of the footpad responsiveness. Recombinant DNA-derived MuIFN-β was less effective than α_1.

Although these observations allow the conclusion that the anti-inflammatory effect of LDH virus is largely due to endogenous interferon, they do not rule out a possible contribution of macrophage dysfunction merely as a result of the virus infection itself. It should be mentioned, however, that in the acute as well as chronic phases of LDH virus infection, only a minor fraction of macrophages is carrying the virus (27). Our observations not only provide a plausible explanation for an old observation, but also reemphasize the need for vigilance on behalf of investigators working with transplantable tumors. Contamination with LDH virus can easily be detected by determination of serum levels of the enzyme lactic dehydrogenase (1). Our observation that interferon is implicated in the effects of LDH virus on host responses suggests that screening for interferon production in the first 2 days after tumor transplantation may also be useful, if not as an alternative, as an additional measure that might in some instances detect contamination with other viruses.

De Maeyer *et al.* (8, 9) have evaluated the effects of systemic interferon on local inflammation, using delayed-type hypersensitivity models. When given in association with the antigen challenge dose, IFN-α,β was found to inhibit the inflammatory response. In contrast, when given in association with the sensitizing dose, interferon caused either stimulation or inhibition, depending on the time schedule used. The pathogenesis of local inflammation elicited by bacterial LPS differs from that of delayed-type hypersensitivity reactions. In its most pronounced form it is known as the Shwartzman phenomenon, which requires a local preparatory injection of LPS followed by an intravenous provocative dose, and which is characterized by the occurrence of vascular damage and thromboembolic processes (28). Involvement of interferon in this reaction has been suggested on the basis of the observations that LPS induces IFN-α,β systematically (29, 30), and that poly(rIrC), another inducer of interferon, can replace the provocative dose of LPS (31). The data presented here indicate that systemic endogenous interferon may indeed affect local reactions to LPS but also that

it has a downregulating rather than a stimulatory effect.

Interferons α and/or β are produced during the acute phase of infections with viruses but also with Gram-negative bacteria. While these interferons are probably instrumental in limiting proliferation of virus and in enhancing phagocytosis and killing of bacteria, our observations suggest that they may also have a role as a modulator of the local inflammatory reaction to the infectious agents. Furthermore, inflammatory reactions to bacterial products, in addition to autoimmune reactivity, are considered to be important elements in the pathogenesis of certain rheumatic diseases. By showing that interferon inhibits LPS-induced inflammation, our study broadens the experimental basis for possible applicability of interferon in such diseases.

This contribution is dedicated to the memory of one of the authors, P. De Somer, Director of the Rega Institute, deceased June 17, 1985.

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1. Rowson KEK, Mahy BWJ. Laboratory methods. In: Gard S, Hallauer C, Eds. Virology Monographs No. 13, Lactic Dehydrogenase Virus. Vienna/New York, Springer-Verlag, pp100–105, 1975.
2. Howard RJ, Notkins AL, Mergenhagen SE. Inhibition of cellular immune reactions in mice infected with lactic dehydrogenase virus. Nature (London) **221**:873–874, 1969.
3. Snyderman R, Cianciolo GJ. Further studies of a macrophage chemotaxis inhibitor (MCI) produced by neoplasms: Murine tumors free of lactic dehydrogenase virus produce MCI. J Reticuloendothel Soc **26**: 453–458, 1979.
4. Stevenson MM, Rees JC, Meltzer MS. Macrophage function in tumor-bearing mice: Evidence for lactic dehydrogenase-elevating virus-associated changes. J Immunol **124**:2892–2899, 1980.
5. Du Buy H, Baron S, Uhlendorf C, Johnson ML. Role of interferon in murine lactic dehydrogenase virus infection, in vivo and in vitro. Infect Immun **8**:977–984, 1973.
6. Evans R, Riley V. Circulating interferon in mice infected with the lactate dehydrogenase-elevating virus. J Gen Virol **3**:449–452, 1968.
7. Asherson GL, Bendinelli M. Immunodepression by viruses: Effect of Friend and Riley viruses on contact sensitivity. J Gen Microbiol **17**:179–188, 1969.
8. De Maeyer E, De Maeyer-Guignard J, Vandeputte M. Inhibition by interferon of delayed type hypersensitivity in the mouse. Proc Natl Acad Sci USA **72**: 1753–1757, 1975.
9. De Maeyer E, De Maeyer-Guignard J. Host genotype influences immunomodulation by interferon. Nature (London) **284**:173–175, 1980.
10. Hauschka TS, Weiss L, Holdridge BA, Cudney TL, Zumpft M, Planinsek JA. Karyotype and surface features of murine TA3 carcinoma cells during immunoselection in mice and rats. J Natl Cancer Inst **47**: 343–359, 1971.
11. Heremans H, Billiau A, Colombatti A, Hilgers J, De Somer P. Interferon treatment of NZB mice: Accelerated progression of autoimmune disease. Infect Immun **21**:925–930, 1978.
12. Zwarthoff EC, Bosveld IJ, Vonk WP, Trapman J. Constitutive expression of a murine interferon alpha gene in hamster cells and characterization of its protein product. J Gen Virol **66**:685–691, 1985.
13. Zwarthoff EC, Van Heuvel M, Mooren ATA, Van Der Korput JAGA, Bosveld IJ, Trapman J. Characterization of the murine interferon-α family. In: Dianzani F, Rossi GB, Eds. The Interferon System. New York, Raven Press (Serono Symposia), Vol 24: pp9–14, 1985.
14. Heine JW, Van Damme J, De Ley M, Billiau A, De Somer P. Purification of human fibroblast interferon by zinc chelate chromatography. J Gen Virol **54**:47–56, 1981.
15. Bosveld IJ, Vonk WP, Helman RACP, Van Vliet PW, De Jonge P, Van Ewijk W, Trapman J. Preparation and properties of monoclonal antibodies against murine interferon-β. Virology **120**:235–239, 1982.
16. Coutelier JP, Van Roost E, Lambotte P, Van Snick J. The murine antibody response to lactate dehydrogenase-elevating virus. J Gen Virol **67**:1099–1108, 1986.
17. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685, 1970.
18. Morrissey JH. Silver stain for protein in polyacrylamide gels: A modified procedure with enhanced uniform sensitivity. Anal Biochem **117**:307–310, 1981.
19. Fauve RM, Hevin B, Jacob H, Gaillard JA, Jacob F. Antiinflammatory effects of murine malignant cells. Proc Natl Acad Sci USA **71**:4052–4056, 1974.
20. Nelson M, Nelson DS. Macrophages and resistance to tumours. I. Inhibition of delayed-type hypersensitivity reactions by tumour cells and by soluble products affecting macrophages. Immunology **34**:277–290, 1978.
21. Nelson M, Nelson DS. Macrophages and resistance to tumors. IV. Influence of age on susceptibility of

mice to anti-inflammatory and anti-macrophage effects of tumor cell products. J Natl Cancer Inst **65:** 781–789, 1980.

22. Norman SJ, Sorkin E. Cell-specific defect in monocyte function during tumor growth. J Natl Cancer Inst **57:** 135–140, 1976.
23. Snyderman R, Pike MC, Blaylock BL, Weinstein P. Effects of neoplasms on inflammation: Depression of macrophage accumulation after tumor implantation. J Immunol **116:**585–589, 1976.
24. Wells JH, Cain WA, Wells RS, Bozalis JR. Suppression of tuberculin and phytohemagglutinin skin tests by large tumors in mice. Int Arch Allergy **47:**362–368, 1974.
25. Wells JH, Pollack VA, Cox CP, Cain WA. Studies of the paw test and tumor-associated immunosuppression in mice. Int Arch Allergy Appl Immunol **56:**391–397, 1978.
26. Cantarow WD, Cheung, HT, Sundharadas G. Modulation of spreading, adhesion and migration of peritoneal macrophages by a low molecular weight factor extracted from mouse tumors. J Reticuloendothel Soc **24:**657–666, 1978.
27. Stueckemann JA, Ritzi DM, Holth M, Smith MS, Swart WA, Cafruny WA, Plagemann PGW. Replication of lactate dehydrogenase-elevating virus in macrophages. 1. Evidence for cytocidal replication. J Gen Virol **59:**245–262, 1982.
28. Wilson GS, Miles A. The mechanism of antibacterial immunity in the normal animal. In: Wilson GS, Miles A, Eds. Topley and Wilson's Principles of Bacteriology, Virology and Immunity. London, Arnold, Vol 2:pp1316–1357, 1975.
29. Ho M. Interferon-like viral inhibitor in rabbits after intravenous administration of endotoxin. Science **146:** 1472–1474, 1964.
30. Stinebring WR, Youngner J. Patterns of interferon appearance in mice infected with bacteria and bacterial endotoxin. Nature (London) **204:**712–715, 1964.
31. Absher M, Stinebring WR. Endotoxin-like properties of poly I. poly C, an interferon inducer. Nature **223:** 715–717, 1969.

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