INHIBITION OF LYMPHOCYTE TRAPPING BY A PASSENGER VIRUS IN MURINE ASCITIC TUMORS: CHARACTERIZATION OF LACTIC DEHYDROGENASE VIRUS (LDV) AS THE INHIBITORY COMPONENT AND ANALYSIS OF THE MECHANISM OF INHIBITION*

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Upon immunization, a variety of events occur in lymphatic tissue. One of the earliest is the trapping of recirculating lymphocytes within the draining lymphatic organs (1). The trap may promote interaction of antigen, macrophages, and specific immunocompetent cells.

Frost and Lance reported that cell-free ascitic fluid (AF)[1] inhibits lymphocyte trapping (2). We confirmed this with fluid from other ascites tumors (3). In this report we show that lactic dehydrogenase virus (LDV), a common passenger in many mouse tumors (4, 5), is a trapping inhibitory component in the tumor AFs. After injection of LDV, the virus replicates quickly and reaches a peak titer in 24 h. It causes a permanent viremia with the most obvious manifestation being an elevation in plasma lactic dehydrogenase (LDH) (4). The virus probably replicates exclusively in macrophages (6). It affects reticuloendothelial system (RES) function (7), causes histological changes in lymphatic tissue (6), and alters some immune responses (8, 9). We have studied the effect of LDV on lymphocyte circulation and have attempted to analyze the mechanism of LDV inhibition of the lymphocyte trap.

Materials and Methods

Animals. Female BALB/c mice, 2–4 mo of age, and female Swiss Webster rats were obtained from Simonsen Farms (Gilroy, Calif.). Specific pathogen-free (SPF) female BALB/c, 3–6 mo of age, were obtained from Dr. R. Kallman (Department of Radiology, Stanford University).

Ascites Tumors. L4946, Ascitic lymphoma, and Krebs-2 tumors were all cultivated in BALB/c mice in the ascitic form. Their derivations are as previously reported (3). The Ehrlich ascitic tumor was kindly donated by Dr. Howard Fieldsteihl of Stanford Research Institute. Cell-free AF was prepared by centrifugation at 1,000 g for 20 min at 5°C.

[1] *Abbreviations used in this paper:* AF, ascitic fluid; Con A, concanavalin A; FCS, fetal calf serum: LDH, lactic dehydrogenase; LDV, lactic dehydrogenase virus; LN, lymph node; NMS, normal mouse serum; PBS, phosphate-buffered saline; PHA-P, phytohemagglutinin P; RES, reticuloendothelial system; SPF, specific pathogen-free; SRBC, sheep red blood cells.

LDV Cultivation and Assay. LDV (supplied by Spencer Hiraki) was cultivated in SPF BALB/c mice. LDH levels, indicative of LDV infection, were assayed by the method of Riley (10).

Cell Suspensions. Cell suspensions were prepared from pooled axillary, brachial, inguinal, and mesenteric lymph nodes of donor mice, and cells were labeled with sodium chromate (^{51}Cr) by procedures previously described (3).

Lymphocyte Trapping Assay. Trapping was measured by the method of Zatz and Lance (1). Trapping was elicited by a dose of 6×10^8 sheep red blood cells (SRBC) injected subcutaneously (s.c.) on the anterior and posterior right flanks. The distribution of 10^7 intravenously (i.v.) injected ^{51}Cr-labeled syngeneic lymphocytes in recipient mice was then determined.

24 h subsequent to labeled lymph node (LN) cell injection brachial, axillary, and inguinal LNs were removed and pooled, preserving bilateral symmetry. The radioactivity within the pooled antigen-draining and the pooled contralateral (control) LNs was measured. The recovery in the pooled antigen-draining LNs of each mouse was expressed as a percentage of the recovery in the pooled control LNs utilizing the standardization procedure described previously (3).

Ether Fractionation of Ehrlich AF. The ether inactivation method of Notkins (11), involving three ether extractions, was used to inactivate LDV. A sham-fractionated preparation was shaken without ether.

Ultraviolet (UV) Inactivation of LDV. LDV-infected AF was sonicated for 30 s to disaggregate the virus particles. A 60-mm petri dish containing 2 ml of a 1:10 dilution of sonicated Ehrlich cell-free AF in 0.15 M NaCl was placed under a (General Electric Co., Cleveland, Ohio) germicidal lamp and received 6,575 ergs/cm^2 per s. The AF was mechanically agitated during exposure. From an inactivation curve, 4 min was the minimal dose selected for complete inactivation of LDV. The treated preparation, in a dose initially equal to 10^7 LDV infectious units, was assayed for LDV and for its ability to inhibit lymphocyte trapping. A sham UV inactivated preparation was also assayed.

Passage of Ehrlich Ascites Tumor in Irradiated Rats. Ehrlich ascites tumor was freed of LDV by passage in animals that do not support LDV replication (10). Swiss Webster rats were lethally irradiated with 900 R, and 2 h later were injected i.p. with 2.4×10^7 Ehrlich tumor cells. 7 days later, the ascites cells were harvested and passaged into SPF BALB/c mice. AF was collected after the first and second BALB/c tumor passage after rat passage and tested for LDV and trapping inhibitory capacity.

In Vitro Incubation of Lymph Node Cells with LDV-Infected L4946 AF. ^{51}Cr-labeled LN cells (1.5×10^8) were incubated in (a) medium 199 + 15% fetal calf serum (FCS) + 30% 0.22 μm millipore-filtered L4946 AF containing 5×10^8 LDV or (b) medium 199 + 15% FCS with occasional shaking in a 37°C water bath. After 30-min incubation, the cells were centrifuged, washed twice, and resuspended for injection. Viabilities of both groups of cells were determined by trypan blue dye exclusion.

Mitogen Stimulation of Lymphocytes in Cultures Containing Cell-Free Ehrlich Ascitic Fluid or Normal Mouse Serum (NMS). Mitogens used for stimulation were Concanavalin A (Con A) (Pharmacia Fine Chemicals, Uppsala, Sweden) and Phytohemagglutinin P (PHA-P) (Difco Laboratories, Detroit, Mich.). In the different test media used, the optimum Con A and PHA-P concentrations for stimulation were always 1 μg and 4×10^{-4} ml stock per culture, respectively. Cells used were: (a) unfractionated LN cells containing 60% Ig negative cells or (b) LN cells enriched for T cells by nylon wool fractionation (12) and containing 90% Ig negative cells. In addition to 5×10^5 cells, at 0 h each well in a sterile, flat-bottomed microculture plate (Falcon Plastics, Oxnard, Calif.) contained either Con A, PHA-P, or medium (RPMI-1640 + 4% FCS) and either 10% millipore-filtered Ehrlich AF (containing 10^7 LDV/culture) or 5% NMS (protein concentration in NMS is twice that of the ascitic fluid). The total culture volume was 0.2 ml. After 48-h incubation at 37°C in a humidified, 5% CO$_2$ atmosphere, 1.0 μCi [^{3}H]thymidine (New England Nuclear, Boston, Mass.) was added to each culture. Cultures were harvested at 72 h on a MASH unit (multiple automatic sample harvester). The cell-incorporated radioactivity remaining on glass filter disks was measured.

Statistics. Results are expressed as means within each group ± SE. The probability (P) values were determined by Student's *t* test.

Results

Lymphocyte Trapping and Its Inhibition by Cell-Free Tumor AF. Groups of mice were injected s.c. in the right flanks with SRBC or phosphate-buffered

TABLE I

Recovery of ^{51}Cr-LN Cells and Percent Trapping in LNs of Mice Injected with Antigen or PBS

	Recovery as percent of injected dose ± SE	
	SRBC ($n = 52$)	PBS ($n = 23$)
Pooled right LNs	4.59 ± 0.16	2.40 ± 0.17
Pooled left LNs	2.00 ± 0.08	2.22 ± 0.16
P value*	$P < 0.001$	$P > 0.2$
Percent trapping‡	130 ± 6	8 ± 4

Mice received 6 × 10^8 SRBC or 0.2 ml PBS s.c. on the right flanks 24 h before i.v. injection of 10^7 ^{51}Cr-LN cells. 24 h after labeled cell infusion, mice were sacrificed, radioactivity in LNs measured, and percent trapping calculated.

* Probability that recovery in pooled right LNs differs significantly from recovery in pooled left LNs.

‡ In this experiment percent trapping was calculated by the following formula:

$$\% \text{ trapping} = \left(\frac{\text{recovery in antigen-draining LNs}}{\text{recovery in contralateral LNs}} \times 100 \right) - 100.$$

saline, (0.1 M, pH 7.2). 24 h later 10^7 ^{51}Cr-labeled LN cells were injected i.v. Antigen-draining and contralateral LNs were excised after 24 h and the radioactivity in the nodes determined. The data in Table I show that a significant increase in lymphocyte localization occurred in the peripheral LNs draining the site of antigen injection. This increase is designated lymphocyte trapping.

To demonstrate inhibition of trapping by tumor AF, groups of mice were injected with antigen alone, antigen and cell-free tumor AF, or antigen and NMS. The mice treated with AF received three 0.5 ml doses of AF 72, 48, and 24 h before labeled cell infusion. The same schedule was used to treat mice with three 0.25-ml doses of syngeneic mouse serum. A dose of 0.25 ml NMS was approximately equal in protein concentration to 0.5 ml AF. All mice received SRBC s.c. on the right flanks 24 h before the labeled cells, and the protocol for assaying trapping was followed. Fig. 1 shows the significant inhibition of trapping observed in mice infused with cell-free AF from four murine tumors: L4946, Ehrlich, Ascitic Lymphoma, and Krebs-2. NMS had no inhibitory effect. These results agree with our previously published results in C3H-Bi mice (3). AF passed through 0.22 μm millipore filters also inhibited trapping (data not given).

Presence of LDV in Cell-Free Tumor AF.   Groups of SPF BALB/c mice were injected i.p. with 0.3 ml of cell-free tumor AF or NMS. 4 days later the mice were tested for increases in LDH levels indicative of LDV infection. The range of LDH levels in plasma of normal mice was 180–1,110 LDH U/ml with a mean of 449 LDH U/ml. Mice injected with fluid from L4946, Krebs-2, Ascitic Lymphoma, and Ehrlich tumors showed an approximately eightfold increase in LDH levels. Mice injected with NMS exhibited normal LDH levels. Thus, each fluid that inhibited trapping was found to contain LDV.
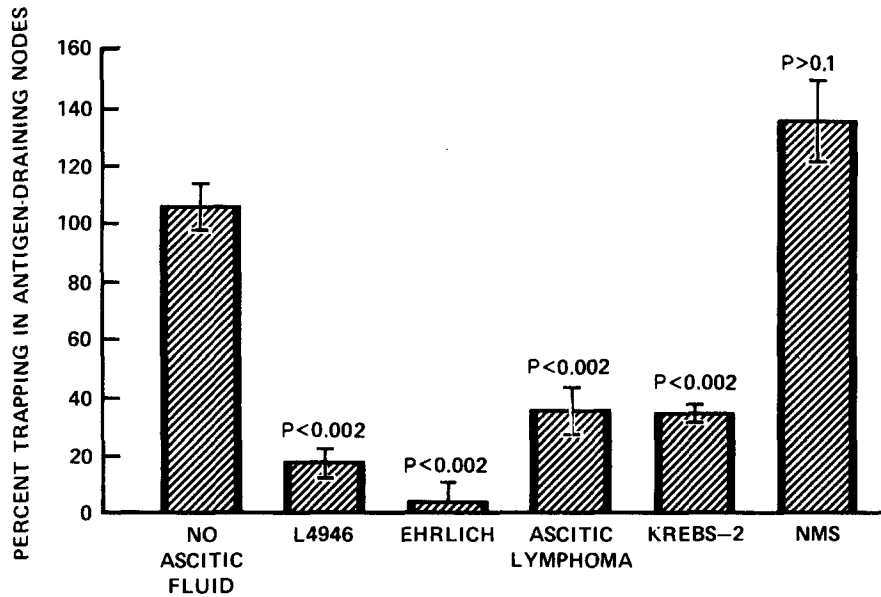
FIG. 1. Inhibition of lymphocyte trapping by cell-free tumor ascitic fluid. Before labeled cells, all mice received SRBC s.c., and some received i.v. infusions of various cell-free tumor ascitic fluids or NMS. Each bar represents the mean of 4–8 mice ± SE. The P values indicate the probability that the percent trapping in i.v. treated mice is not significantly different from that in control, untreated mice.

Dose Response of Trapping Inhibition and LDV Infection by AF. Concurrently with the trapping eliciting dose of SRBC, groups of mice were injected i.v. with dilutions of cell-free Ehrlich AF in medium 199 or with medium alone. Plasma LDH levels and trapping were assayed as described.

Fig. 2 illustrates the dose response of LDV infectivity and inhibition of trapping by Ehrlich AF. The group of mice that received 50-5 × 10^{-7} ml of AF exhibited a significant ($P < 0.001$) inhibition of trapping as well as a significant ($P < 0.001$) rise in plasma LDH levels indicative of LDV infection. 6 of the 10 mice that received 5 × 10^{-8} ml of AF exhibited significantly ($P < 0.002$) elevated levels of LDH. With only one exception, the mice that exhibited elevated LDH levels were inhibited from trapping, while the mice with normal LDH levels had normal trapping levels. Thus the minimum dose of AF required for trapping inhibition correlates with the minimum dose of AF required to elevate LDH levels in recipient mice.

Effect of Procedures that Eliminate LDV Upon the Trapping Inhibitory Capacity of Tumor AF. To clarify if LDV was the inhibitory component in tumor AF, ether fractionation and UV irradiation of infected AF and rat passage of the infected tumor were used to eliminate infectious LDV. SPF BALB/c mice which received a trapping eliciting dose of SRBC simultaneously received 0.3 ml of ether- or sham-fractionated Ehrlich AF or UV- or sham-irradiated Ehrlich AF i.v. Other groups of mice received three doses of 0.5 ml AF from rat-passaged or nonrat-passaged Ehrlich tumor i.v. at 48, 24, and 0 h before s.c. SRBC injection. Trapping was assayed and LDH levels measured.
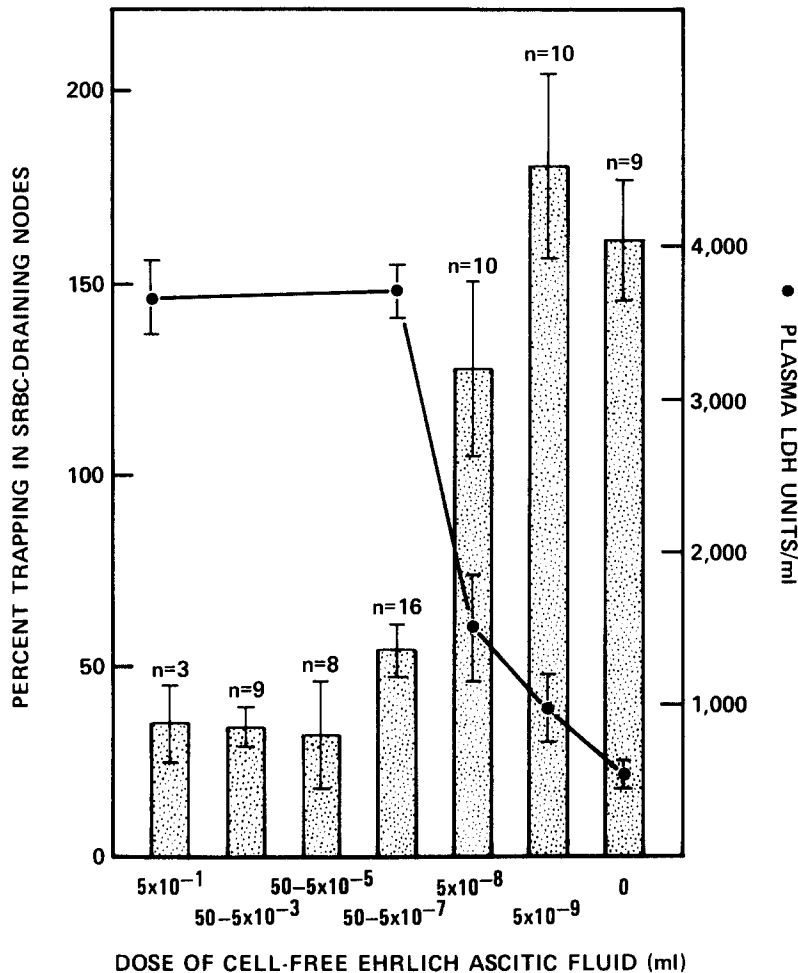
FIG. 2. Dose response of trapping inhibition and LDV infection with cell-free Ehrlich ascitic fluid. The percent trapping (bars) ± SE and the LDH enzyme levels (●) ± SE are plotted as a function of the dose of ascitic fluid injected.

As shown in Fig. 3 A mice injected with ether-treated AF exhibited as much trapping as control mice that received no AF. Trapping in mice that received sham-fractionated AF was depressed. Ether-treated AF no longer had the capacity to elevate plasma LDH levels in recipient mice.

The effect of UV-irradiated AF upon lymphocyte trapping is illustrated in Fig. 3 B. Only 1 of the 13 mice that received UV-treated AF exhibited significant inhibition of trapping. This mouse had an elevated plasma LDH level (3,270 LDH U/ml) indicative of LDH infection. Mice that received sham-irradiated AF were infected with LDV and were significantly inhibited from trapping. Thus, UV irradiation abolished the trapping inhibitory capacity of tumor AF, and this appeared to be dependent upon the inactivation of LDV.

As shown in Fig. 3 C the percent trapping in mice treated with rat-passaged Ehrlich tumor AF was not significantly different from that in mice that received
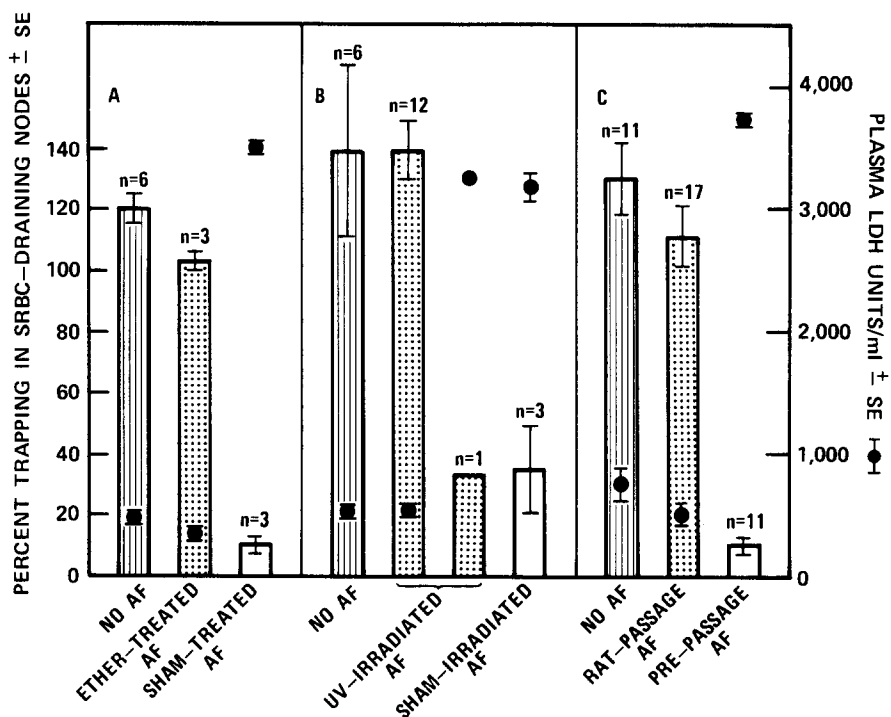
FIG. 3. Test of the trapping inhibitory capacity of tumor AF after various treatments to eliminate infectious LDV. (A) Effect of ether-treated tumor AF upon lymphocyte trapping. Percent trapping (bars) and plasma LDH levels (●) were assayed in mice that received only SRBC s.c. or SRBC and i.v. infusion of ether-treated or sham-treated AF. (B) Effect of UV-irradiated AF upon trapping. Mice received only SRBC s.c. or SRBC s.c. and UV-irradiated or sham-irradiated AF i.v. The AF dose contained 10^7 infectious units before UV treatment. (C) Effect of AF from a rat-passaged tumor upon trapping. Mice received only SRBC s.c. or SRBC s.c. and AF from rat-passaged or nonrat-passaged Ehrlich ascitic tumor i.v.

no AF. Mice treated with AF from the same tumor before rat passage were inhibited in trapping.

To recapitulate, the elimination of infectious LDV by dilution, ether-treatment, UV irradiation, and rat passage of the infected tumor abolished the trapping inhibitory capacity of tumor AF. LDV appears to be the inhibitory component in our tumor AFs. Experiments performed with LDV in plasma of nontumor-bearing mice indicated that LDV, dissociated from tumors, could inhibit trapping (see Fig. 4).

Effect of Time of LDV Administration Upon Inhibition of Trapping. Trapping occurs soon after antigen reaches the draining lymphatic tissue. Within 15 min after i.v. injection and within 24 h after s.c. injection, trapping can be measured in the spleen and lymph node, respectively (1, 13). To determine if LDV could inhibit a trapping response already initiated, groups of mice were injected i.v. with 10^6 LDV 0, 6, 12, 24, 36, 40, or 45 h after s.c. injection with SRBC. Trapping was assayed. Fig. 4 shows the increases in trapping seen when LDV is given at increasing time intervals after SRBC injection. When LDV was injected up to 12 h after SRBC only 40% trapping was observed. If LDV

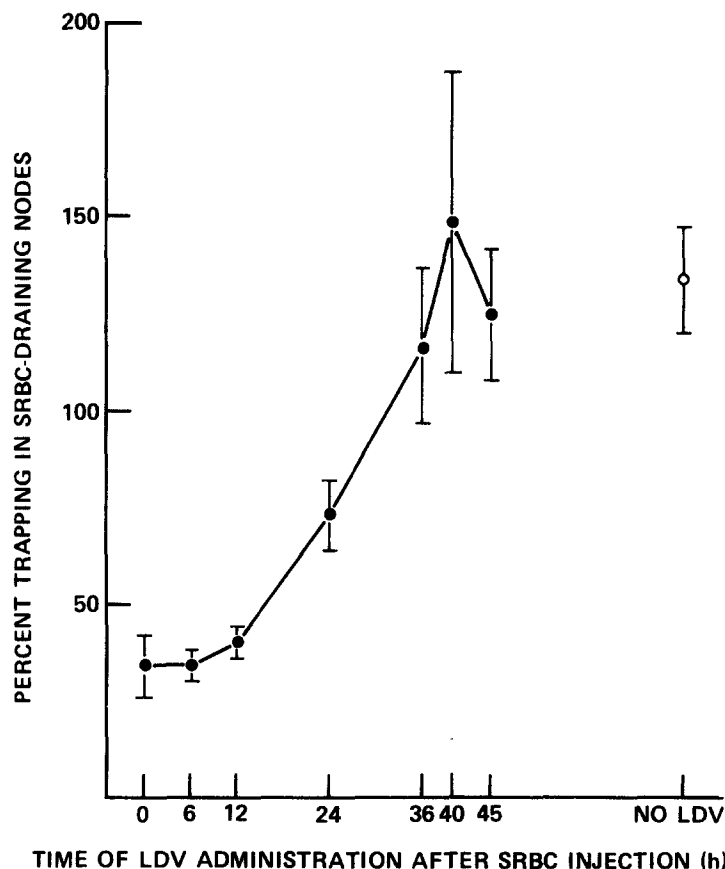TIME OF LDV ADMINISTRATION AFTER SRBC INJECTION (h)

FIG. 4. Effect of time of LDV administration upon inhibition of trapping. The amount of trapping in LNs of mice that received SRBC s.c. is plotted as a function of time of i.v. infusion with 10⁷ LDV in plasma of nontumor-bearing mice. The percent trapping in mice that received no LDV is also shown (O).

was not given until 24 h after SRBC 73% trapping was observed. Mice receiving LDV 36–45 h after antigen had comparable levels of trapping as control mice. Thus LDV was not as efficient in inhibiting trapping when given late after antigen injection. This suggests that to inhibit trapping LDV must be present before trapping is initiated and/or some replication of the virus must occur.

Trapping Inhibition in Mice Acutely or Chronically Infected with LDV. Mice acutely infected with LDV exhibit elevated plasma LDH levels (8), impaired RES carbon clearance (7), and a depletion of lymphocytes in the T-cell dependent areas of lymphatic tissues (6). Except for the elevated LDH levels, these effects are transient (6–8). To determine if the inhibition of trapping occurs in only the acute or in both acute and chronic infections, SRBC were injected s.c. into mice that had received 10⁶ LDV i.v. 9 days previously (chronic LDV infection) or that were to receive 10⁶ LDV i.v. concurrently with antigen (acute LDV infection). Trapping was assayed. Mice ($n = 52$) not infected with LDV exhibited $130\pm6\%$ trapping, while mice ($n = 13$) acutely infected exhibited

TABLE II
*Effect of Infusion of LDV-Infected Tumor Ascitic Fluid, LDV-Infected Plasma, or
NMS upon Lymphocyte Localization in Nonantigen Draining LNs*

i.v. treatment*	n	Recovery ± SE	P value‡
		%	
None	77	2.12 ± 0.06	—
LDV-infected Krebs-2 AF	6	3.08 ± 0.24	<0.001
LDV-infected L4946 AF	12	3.03 ± 0.16	<0.01
LDV-infected Ehrlich AF	32	3.18 ± 0.08	<0.001
LDV-infected plasma (day −1)	10	3.39 ± 0.20	<0.001
LDV-infected plasma (day −10)	3	2.46 ± 0.34	>0.2
Ether-treated Ehrlich AF	2	1.90 ± 0.08	>0.5
Rat-passaged Ehrlich AF	18	1.60 ± 0.07	<0.001
LDV-free L4946 AF	6	2.60 ± 0.29	>0.1
NMS	4	1.74 ± 0.16	>0.1

* Mice received doses of 3 × 0.5 ml LDV-infected Krebs-2 or L4946 AF, 3 × 0.5 ml or 1 × 0.5 ml LDV-infected Ehrlich AF, 0.1 ml LDV-infected plasma, 0.3 ml ether-treated AF, 3 × 0.5 ml rat-passaged tumor AF, 3 × 0.5 ml LDV-free L4946 AF, or 3 × 0.25 ml NMS at 72, 48, and 24 h before or at 24 h before labeled LN cells. One group of mice received LDV-infected plasma 10 days before labeled LN cells. 24 h after labeled cell injection, LNs were excised, and the recovery of cells in pooled unilateral LNs was measured.

‡ Probability that recovery of labeled cells in treated mice is not significantly different from recovery in control untreated mice.

36±6% trapping, a significantly (*P* < 0.001) lesser amount. No inhibition of trapping was observed in mice (*n* = 3) chronically infected with LDV. These mice exhibited 117±9% trapping. Both acutely and chronically infected mice exhibited levels of LDH significantly above control levels.

Effect of Infusion of Cell-Free Tumor AF, LDV-Infected Plasma, or NMS Upon Lymphocyte Localization in Nonantigen-Draining LNs. In our assays for lymphocyte trapping we observed that mice receiving LDV-infected fluids, but not SRBC, exhibited an increased labeled cell localization in LNs compared to mice not receiving LDV. The observations are presented here. The recovery of labeled lymphocytes in LNs of mice that had received an i.v. injection of LDV-infected tumor AF, LDV-infected plasma, LDV-free tumor AF, or NMS before labeled cell infusion was compared to the recovery of lymphocytes in mice receiving no i.v. treatment. The recovery in LNs of mice that had received i.v. injection of LDV-infected plasma 10 days previous to labeled cell injection was also compared to the recovery in LNs of mice that received no i.v. treatment. The assayed LNs did not drain the site of SRBC injection.

The results demonstrate (Table II) that a very significant increase in labeled cell recovery occurs in the unilaterally pooled inguinal, axillary, and brachial LNs of mice receiving LDV-infected Krebs-2, L4946, and Ehrlich cell-free AFs and in mice receiving LDV-infected plasma shortly before injection of labeled lymphocytes. The cell localization in mice that received virus-infected plasma 10 days before labeled cells, however, was not altered. LDV-free AFs did not cause any increase in lymphocyte localization in peripheral nodes. The cell recovery in mice infused with rat-passaged Ehrlich AF was, however, significantly less than the recovery in control mice.
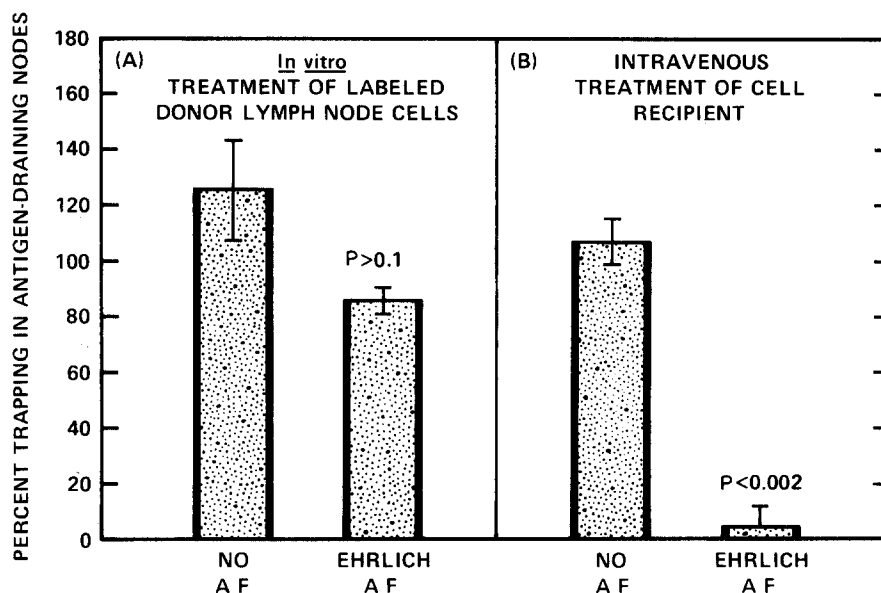
FIG. 5. Effect of in vitro incubation with LDV-infected AF upon the ability of donor lymphocytes to be trapped. (A) Labeled cells were incubated in vitro with 30% cell-free Ehrlich AF or with medium alone before i.v. transfer into mice that had received SRBC s.c. Trapping in the SRBC-draining nodes was subsequently measured. (B) Trapping was assayed in mice treated as described in Fig. 1.

Effect of Increasing Labeled Lymphocyte Dose Upon Labeled Cell Recovery in Antigen-Draining LNs. Inasmuch as infusion with LDV-infected fluids resulted in increased retention of labeled cells in peripheral LNs, we tested to see if the inhibition of SRBC-induced trapping was due to a saturation of the antigen-draining LNs with lymphocytes. Increasing numbers of labeled lymphocytes were injected i.v. into mice that 24 h previously had received a trapping eliciting dose of SRBC together with an i.v. injection of 0.3 ml cell-free LDV-infected Ehrlich AF. When 10^6, 10^7, or 10^8 labeled lymphocytes were injected, 2.01×10^4, 2.03×10^5, and 1.37×10^6 cells, respectively, were recovered in the antigen-draining LNs. Since the cell recovery is nearly linear, we conclude that the inhibition of SRBC-elicited trapping is not due to LN saturation produced by the increased lymphocyte localization in LNs after LDV treatment.

Effects of In Vitro Incubation of Labeled Donor Cells with LDV-Infected Cell-Free Ascitic Fluid. LDV may affect circulating cells so as to make them less able to be trapped. To test this possibility, labeled LN cells were incubated with 30% LDV-infected Ehrlich AF or with medium alone for 30 min at 37°C. Cells were washed twice, their viability shown to be unaffected by treatment with AF, and injected i.v. into mice that had 24 h previously received SRBC s.c. As shown in Fig. 5 the amount of trapping that occurred when labeled donor LN cells were incubated with LDV-infected tumor AF was not significantly different from that in mice infused with cells incubated in medium alone. This is in contrast to the very significant inhibition of trapping observed when the AF was administered i.v. The slight amount of inhibition observed with in vitro incubation of donor

TABLE III

Mitogen Stimulation of Lymphocytes with LDV-Infected AF or NMS in the Culture Medium

Culture medium additive	Mean cpm × 10⁴/culture ± SE			
	Con A		PHA-P	
	LN Cells	T-cell enriched LN cells	LN cells	T-cell enriched LN cells
10% Ehrlich AF, (10⁷ LDV/culture)	14.15 ± 2.55 (6)*	8.13 ± 4.21 (3)	3.73 ± 1.18 (6)	3.35 ± 1.88 (3)
5% NMS‡	14.77 ± 1.24 (6)	11.82 ± 2.78 (3)	2.01 ± 0.41 (6)	3.31 ± 1.34 (3)
P value§	>0.5	>0.2	>0.1	>0.5

Unstimulated cultures contained approximately 125 cpm.
* Number of experiments with 3 or 6 replicates/experiment.
‡ Medium with 5% NMS had an equivalent protein concentration to medium with 10% Ehrlich AF.
§ Probability that stimulation in cultures containing LDV-infected AF is not significantly different from cultures containing NMS.

cells with AF is probably due to transfer of some virus with the cells at a considerable time after injection of antigen that elicited trapping.

Mitogen Stimulation of Lymphocytes with LDV-Infected Cell-Free Tumor AF or NMS in the Culture Medium. LDV may inhibit trapping by preventing induction of the trap. Macrophages and T cells have each been implicated in inducing the lymphocyte trap (13, 14). To determine if the LDV-infected AF might inhibit trapping via the T cell, we studied its effect on T-cell responsiveness in vitro.

Stimulation by the mitogens, Con A, and PHA was used as our assay of T-cell reactivity. Table III shows the data from experiments of PHA-P and Con A stimulation of unfractionated LN cells and T cell-enriched LN cells. With both mitogens and with both populations of cells, the stimulation with LDV-infected AF in the culture medium was not significantly different from the stimulation with NMS in the culture medium.

Neither viability nor in vitro reactivity of T cells to mitogens was influenced by the presence of LDV. Thus, LDV does not appear to inhibit lymphocyte trapping by directly interfering with T-cell function.

Discussion

We extended the observation (2) that cell-free fluid from two ascitic tumors could inhibit lymphocyte trapping to three additional ascitic tumors in C3H-Bi mice (3). The results we report here in BALB/c mice support these previous observations.

Our results implicate LDV as the trapping inhibitory component in tumor AF: (a) the dose response endpoint for trapping inhibition by tumor AF correlates with the endpoint for LDV infectivity, and (b) elimination of infective LDV by ether-fractionation, UV inactivation, and rat-passage of the ascitic tumor eliminates the capacity of the AF to inhibit lymphocyte trapping. To exclude the possibility that a tumor-induced trapping inhibitory factor exists which is inactivated by treatments used to eliminate LDV, an effective neutralizing antibody is required. Total antibody neutralization of LDV is very difficult (15), and the presence of a few residual infectious units in an antibody-neutralized preparation would cause trapping inhibition.

LDV is frequently found in transplanted tumors (4, 5). This virus has not been found to replicate in tumor cells or to be oncogenic (16, 17), but is a passenger virus transmitted with tumor cells and oncogenic virus pools (10).

LDV is difficult to characterize by standard methods. Although most of the virus is recovered in the pellet, LDV has a nonsedimentable infectious component after centrifugation at 105,000 g for 4 h (18). The virus can have residual infectivity after 60°C incubation for 40 min (10). G-200 gel filtration of this virus has not been completely successful due to a minor fraction which tails from the major excluded fraction (10). Any residual virus in a fraction after a characterization step may cause misleading results. We have shown that the few infectious units in a 5×10^{-8} dilution of AF were capable of significantly inhibiting trapping. This is probably explained by the extremely rapid replication time of LDV, estimated at 30 min in vivo (10). Because of these properties, LDV may evade recognition and cause ambiguous results in procedures used to characterize immunosuppressive components in tumor fluids.

LDV has been thought to be a benign virus in that it does not affect mouse life span nor cause overt disease. However, LDV does have effects on the immune system (6–8), and our results demonstrate that acute infection with LDV affects the localization of circulating lymphocytes. A possible mechanism of LDV-induced alteration of lymphocyte circulation is that LDV itself may elicit a trapping response in all the LNs. Smith and Morris have reported that viruses can elicit lymphocyte trapping (19). However, the profound changes observed in the histology of LNs early after LDV infection (6) do not support the existence of a systemic lymphocyte trap. In the first 48 h after viral infection a depletion, rather than an increase in number, of lymphocytes occurs in the thymus-dependent areas of the spleen and the paracortical regions of LNs. This may be caused by a cytotoxic degeneration of T cells and their phagocytosis by macrophages (6).

The depletion of cells in T-dependent areas may account for the alteration in lymphocyte localization after LDV inoculation. The increased recovery of labeled donor cells in peripheral LNs may be due to a greater sink for lymphocytes in the depleted areas and to continued cytotoxicity of incoming T lymphocytes in these regions. The phagocytosis of degenerating labeled cells by macrophages may localize the label in the lymphatic organ.

How does LDV inhibit SRBC-elicited lymphocyte trapping? Since in vitro treatment of donor cells with LDV-infected AF did not significantly inhibit trapping, we believe LDV does not inhibit trapping by altering the circulating cells so that they cannot be trapped.

LDV may inhibit trapping by affecting the induction of the lymphocyte trap. Although most evidence suggests that the macrophage is the cell that induces the trap (13, 20), some evidence suggests that the T cell may be an auxiliary cell to the macrophage (14). The evidence does not clearly implicate the macrophage or the T cell as the site of LDV inhibition of trapping. The following observations support the hypothesis that LDV inhibits trapping via the macrophage. LDV appears to replicate exclusively in the macrophage (6), and the first 48 h after LDV infection pyknotic phagocytic reticular cells are observed in lymphatic

tissue (6). Carbon clearance by the RES is impaired the first few days after infection and returns to normal after a week (7). Correspondingly, our results demonstrate that trapping is inhibited 2 days after LDV infection but not at 10 days after infection.

Although LDV has not been found to replicate in lymphocytes (6), and LDV does not appear to affect T-cell viability nor reactivity to mitogens in vitro, the virus may indirectly inhibit trapping via the T cell. In vivo, LDV infection results in a transient T-cell depletion possibly caused by a cytotoxic factor released by LDV-infected macrophages (6). If the T cell is involved in the induction of the trap, the temporary loss of a T-cell population in an antigen-draining LN could explain how trapping is inhibited.

Trapping inhibition by LDV may be partially or totally due to the changes in systemic localization of injected lymphocytes. The increased localization of labeled cells in all LNs during an acute LDV infection may draw lymphocytes out of the circulating pool available for being trapped in the SRBC-draining LNs.

If trapping potentiates immune responses, LDV, which diminishes trapping, might be expected to be immunosuppressive. LDV prolongs the retention of allogeneic skin grafts (9), potentiates the growth of tumors (21, 22), and increases or decreases antibody responses depending upon the time of LDV inoculation (8). Several immunosuppressive properties of uncharacterized fractions of tumors or tumor AFs (2, 23–26) could be due to LDV.

LDV has a limited host range: the mouse. It may serve as a model for viruses in man which appear relatively benign, but may have the insidious capacity to alter the immunological capacity of the host.

Summary

Cell-free fluid from several ascites promoting tumors inhibits lymphocyte trapping. Lactic dehydrogenase virus (LDV), a common passenger virus in many mouse tumors, was found to be a trapping inhibitory component in these fluids. Procedures used to eliminate infective LDV, such as dilution, passage of the tumor through irradiated rats, ether fractionation, and ultraviolet (UV) irradiation abolished the trapping inhibitory capacity of the fluids. LDV, dissociated from tumors, was inhibitory. Lymph nodes in mice with acute, but not chronic, LDV infections were inhibited from trapping. LDV does not appear to inhibit the capacity of circulating cells to be trapped, and as measured by mitogen responsiveness, the virus does not directly interfere with T-cell function. LDV may inhibit trapping by indirectly affecting the T cell or directly affecting the macrophage in which it replicates. The known characteristics of LDV infection may explain a number of reported immunosuppressive attributes of tumor-associated ascitic fluids.

References

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