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XII. Inhibition of Activated T Cells by Newcastle Disease Virus*

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Natural and vaccine-induced virus infections often are associated with depression of delayed-type hypersensitivity (DTH),[1] T-helper cell function, and the in vitro proliferative response of lymphocytes to antigens and phytohemagglutinin (1–3). The underlying mechanisms are complex and have not yet been fully elucidated. However, in certain myxo- and paramyxovirus infections, unresponsiveness seems to be related, in part, to virus-induced changes in T-lymphocyte traffic (4, 5). When injected intravenously (i.v.) into rats or mice, Newcastle disease virus (NDV) causes transient lymphocytopenia and depletion of lymphocytes in the thymus-dependent areas of lymphoid tissue (6). Lymphocyte output from the thoracic duct is depressed, but recovery occurs within 72 h after virus injection. These changes are related in some way to adsorption of NDV to recirculating T cells. It is in keeping with this view that lymphocytopenia occurs in mice injected with ultraviolet-irradiated NDV (UV-NDV) which cannot achieve a complete replication cycle in embryonated eggs (6).

The results of the current investigation confirm and extend these observations by showing that NDV has a transient influence on T-cell traffic into lymph nodes and inflammatory foci. Cell migration is distorted after brief exposure of thoracic duct lymphocytes (TDL) in vitro to either infectious NDV (I-NDV) or UV-NDV before i.v. injection into syngeneic rats. But I-NDV has another lasting effect upon antigen-activated T cells which cannot be duplicated by irradiated virus. It is revealed in the ability of infectious virus to abrogate the protective immunity and DTH transferred by TDL obtained from rats infected with *Listeria monocytogenes*. The inhibitory influence of I-NDV is reflected in permanent exclusion of labeled S-phase lymphocytes from *Listeria*-induced exudates. These effects are observed after treatment of the cells with low virus multiplicities. The mechanism has not been determined; however, there are reasons for thinking that activated T cells are especially vulnerable to I-NDV because they alone provide the appropriate milieu for virus replication (7).

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[1] *Abbreviations used in this paper:* BSS, balanced salt solution; DTH, delayed-type hypersensitivity; FCS, fetal calf serum; I-NDV, infectious NDV; LA, lymphocyte agglutination; LMA, *L. monocytogene* antigen; NANA, *N*-acetyl-neuraminic acid; NDV, Newcastle disease virus; PBS, phosphate-buffered saline; PEC, peritoneal exudate cells; TDL, thoracic duct lymphocytes; UV-NDV, ultraviolet-irradiated NDV; VSV, vesicular stomatitis virus.

Materials and Methods

The influence of NDV on the tissue disposition and function of lymphocytes was measured by different but complementary techniques. First, TDL taken from rats infected with *L. monocytogenes* were radioactively labeled. The cells were then incubated in vitro with NDV to allow infection of susceptible cells and/or destruction of membrane receptors for NDV. Free virus was removed by washing before the cells were infused into similarly infected recipients. Localization of labeled donor lymphocytes in lymph nodes and in peritoneal exudates induced by killed *L. monocytogenes* was measured radiometrically. Second, unlabeled TDL, incubated with NDV under identical conditions, were compared with untreated TDL in several assays of T-cell function.

Animals. Male and female (Lewis × DA)F_1 hybrid rats were used in most experiments; however, male Lewis rats were used in one experiment involving transfer of TDL into irradiated recipients.

Cells and Tissues. TDL were collected for 16–24 h into heparinized Ringer's solution without added antibiotics. Before labeling or infusion into recipient rats, the cells were washed once with Hanks' balanced salt solution (BSS) containing 1% fetal calf serum (FCS). Peritoneal exudate cells (PEC) were obtained from rats stimulated intraperitoneally (i.p.) with 50 μg of alcohol-killed *L. monocytogenes* (8).

Various lymph nodes were removed for radiometric analysis. The brachial and axillary ("somatic") nodes were combined into a single pool. Mesenteric lymph nodes were assayed alone or in a pool of "visceral" nodes. These included the mesenteric, cecal, renal, and hepatic nodes.

Radioactive Labeling of TDL. Lymphocytes in the DNA synthetic (S) phase of the mitotic cycle were labeled by incubating TDL in vitro for 1 h in medium 199 containing 5% FCS, 1 U of heparin/ml, and [^{14}C]thymidine (40–60 mCi/mmol; New England Nuclear, Boston, Mass.) at a final concentration of 0.5 μCi/ml. The procedure labels 30–60% of immunoblasts; small lymphocytes do not become labeled.

Two procedures were used to label small lymphocytes preferentially. In one experiment, prospective donors of TDL were given a single pulse of vinblastine (5 μg/g), a drug that is selectively toxic for immunoblasts (9). Cells collected during the first 12 h of lymph drainage were incubated for 1 h in medium containing 2 μCi/ml of [5-^{3}H]uridine (20 Ci/mmol, New England Nuclear). This procedure labels approximately 75% of small lymphocytes, but only T cells become heavily labeled (10, 11). In another experiment, TDL were obtained from rats 15 days after the animals had been given the last of 14 daily injections of [^{3}H]thymidine (3 Ci/mmol). During the "rest" interval, radioactivity becomes concentrated in small lymphocytes, both T and B, which have a potentially long life-span.

Recipients of labeled TDL were injected subcutaneously (s.c.) with 1 ml of 0.85% sodium chloride containing 25 μg of the appropriate unlabeled nucleoside(s). In addition, the animals were offered "cold" thymidine (10^{-3} M) or cold thymidine and cold uridine (10^{-3} M) in their drinking water beginning 48 h before cell transfer.

Measurement of Radioactivity. 10^8 TDL, PEC, or lymph nodes from individual rats were extracted with 15 ml of ice-cold TCA. The insoluble pellet was washed twice with cold TCA. To each sample, 0.1 ml of hydrogen peroxide was added. The bleached pellet was then digested for 18 h at 50°C with 0.5–1.0 ml of normal calf serum solubilizer (Amersham/Searle Corp., Arlington Heights, Ill.). Thereafter the sample was neutralized with 0.2 ml of glacial acetic acid, 10 ml of PCS scintillant (Amersham/Searle Corp.) was added and the radioactivity measured in a Beckman liquid scintillation system (Beckman Instruments, Inc., Fullerton, Calif.). The radioactivity in PEC or lymph nodes of rats infused with labeled TDL was expressed as percent counts per minute originally present in the donor inoculum.

Autoradiography. Air-dried smears of TDL were fixed in methanol and prepared as autoradiographs by a procedure described elsewhere (9).

NDV. The California strain of NDV was grown for 36–48 h in the allantoic cavity of 10-day-old embryonated eggs (Spafas, Inc., Norwich, Conn.). Infected allantoic fluid was clarified by centrifugation (500 *g* × 20 min). The virus was then concentrated by centrifuging the fluid for 2 h at 100,000 *g*. Concentrated virus was stored up to 3 mo at −70°C without loss of activity as measured by lymphocyte agglutination, egg infectivity, or its capacity to destroy lymphocyte receptors for NDV.

Lymphocyte Agglutination. Serial twofold dilutions of NDV in 0.05 ml of PBS were dispensed into the wells of microtiter plates (Cooke Laboratory Products Div., Dynatech Laboratories Inc., Alexandria, Va.). An equal volume of phosphate-buffered saline (PBS) containing 3–12 × 10^6 TDL was added to each well. After vigorous agitation, the cells were allowed to settle for 90 min at 4°C. The end point was expressed as the highest dilution of virus in which the TDL formed a uniform carpet on the floor of the well. A lymphocyte agglutination (LA) unit was arbitrarily defined as the concentration of virus which could agglutinate 10^7 TDL under the conditions described.

Egg Infectivity. The embryo infectivity dose (EID_{50}) for embryonated eggs was calculated by the method of Reed and Muench (12).

Ultraviolet Irradiation. 0.8 ml of NDV, diluted 1:8 in PBS, was dispensed into 35 × 10 mm Falcon dishes (BioQuest Div., Becton, Dickinson & Co., Cockeysville, Md.). The dishes were supported on a bed of crushed ice, placed on a rotating platform, and exposed for 10 min to UV irradiation from two G-15T8 germicidal lamps (Norelco, Lynn, Mass.). The latter, operating at a distance of 20 cm, delivered 25.8 × 10^3 ergs/mm^2 to the fluid-air interface. This large dose of irradiation inhibited replication of NDV in eggs. However, irradiation did not impair the capacity of the virus to agglutinate TDL or its ability to destroy membrane receptors for NDV.

Incubation of Lymphocytes with NDV. TDL were washed once in BSS containing 1% FCS and resuspended at a concentration of 33 × 10^6 cells/ml in PBS containing NDV. The cells were incubated for 30 min at 4°C. Thereafter they were sedimented by centrifugation (500 *g* × 10 min), resuspended in virus-free medium 199 containing 1% FCS, and incubated an additional 60 min at 37°C. Finally, the TDL were centrifuged from the medium and resuspended for i.v. transfer into recipient rats. When sugar was added to the incubation mixture, the homologous sugar also was included in the medium with which the cells were washed.

Sugars. *N*-acetyl-neuraminic acid (NANA) was obtained from Böehringer & Soehne, Mannheim, Germany. L-fucose, D-galactose, D-mannose, *N*-acetyl-glucosamine, and *N*-acetyl-galactosamine were purchased from Sigma Chemical Co., St. Louis, Mo.

L. monocytogenes. *L. monocytogenes*, strain EGD, was used for immunization and challenge. A suspension of organisms prepared from a trypticase soy broth culture of infected mouse spleen was stored in liquid nitrogen and recovered as needed. A soluble (*L. monocytogenes* antigen (LMA)) was prepared from the organisms by a procedure described elsewhere (13).

Antimicrobial Immunity. The immunity engendered in rats by S.C. injection of approximately 5 × 10^6 living *L. monocytogenes* was measured indirectly, in terms of the specific resistance conferred on normal rats by an i.v. injection of 2 × 10^6 "immune" TDL/g of body weight (14).

DTH. Test subjects were given a single i.v. pulse of [^{3}H]thymidine, 0.5 μCi/g. 24 h later, 1 μg of LMA in 0.02 ml of saline and 0.02 ml of saline diluent were injected intradermally into the pina of the right and left ears, respectively. 24 h later still, uniform biopsies (13) were removed from the injection sites. The ratio of counts per minute in the right ear biopsy to that in the left ear biopsy was expressed as a "DTH index."

Graft-vs.-Host Reaction. The responsiveness of Lewis TDL to DA alloantigens was measured in vivo by a thymidine incorporation technique (15).

Statistical Methods. Differences between groups were evaluated by analysis of variance and the Q test (16).

Results

Influence of I-NDV on the Tissue Disposition of TDL. [^{14}C]thymidine was used to label a portion of immunoblasts in the thoracic duct lymph of 6-day *Listeria*-infected donors. Small lymphocytes in the lymph of similarly infected rats were labeled with [5-^{3}H]uridine, as described in the Material and Methods. The two labeled cell populations were then combined into a single pool, dispensed into culture flasks, and incubated briefly in medium containing various concentrations of I-NDV. Immediately thereafter the cells were washed and 2 × 10^8 were transferred i.v. into each of 30 *Listeria*-infected recipients. The TDL were infused approximately 1 h after the recipients had been stimulated i.p. with 50 μg of killed *L. monocytogenes*. All were sacrificed 24 h after transfer;

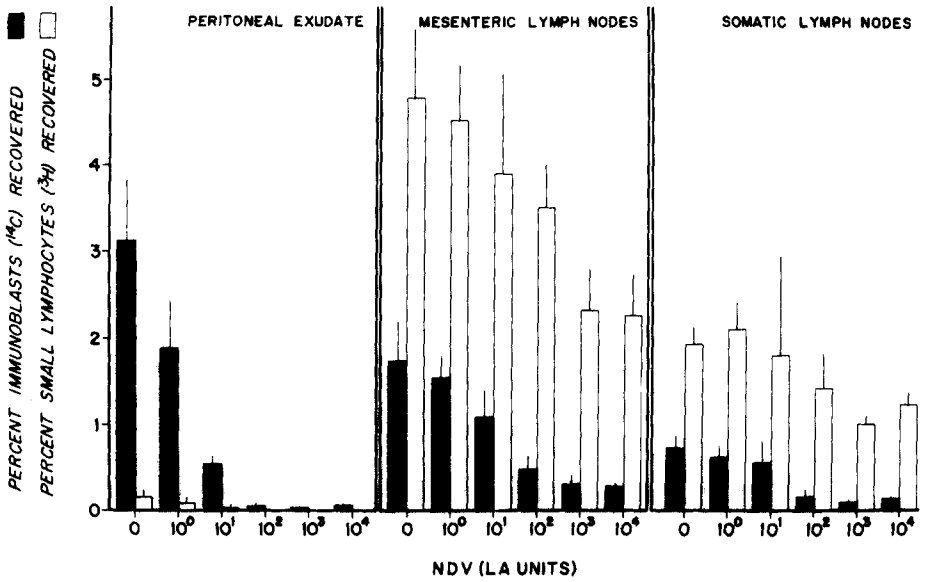

FIG. 1. Cell-associated radioactivity in peritoneal exudates and lymph nodes from *Listeria*-infected rats 24 h after the animals had been infused with a mixture of [^{14}C]thymidine-labeled immunoblasts and 5-^{3}H-labeled small lymphocytes prepared from the thoracic duct lymph of similarly infected donors. The labeled cells were incubated in vitro with I-NDV immediately before transfer. Whereas the virus readily diverted labeled donor immunoblasts from exudates, relatively high concentrations were required to impede the accumulation of labeled small lymphocytes in lymph nodes. Means of 5 ± SD.

PEC were harvested and two groups of lymph nodes removed for radiometric analysis.

The results are shown in Fig. 1, where it can be seen that ^{14}C-labeled donor immunoblasts localized in substantial numbers in the exudates, whereas both labeled immunoblasts and ^{3}H-labeled small lymphocytes were found in lymph nodes. The influx of labeled immunoblasts into exudates was readily inhibited by I-NDV. Witness the reduction in cell-associated ^{14}C after treatment of donor TDL with only 1 LA unit of virus. Higher virus multiplicities were required to impede the accumulation of labeled lymphocytes in lymph nodes. Indeed, a significant ($P < 0.05$) reduction in cell-associated ^{3}H in both mesenteric and somatic nodes was observed only after treatment with 10^3 or 10^4 LA units.

Inhibition of Listeria-Immune Lymphocytes by I-NDV. The specific mediators of resistance to *L. monocytogenes* have been identified as activated T cells (17–19). Since I-NDV can impede the exudate-seeking capacity of labeled immunoblasts, and as the latter are mainly T cells (19), it was logical to postulate that protective cells in the thoracic duct lymph of *Listeria*-immune rats would also be inhibited by virus. The results in Fig. 2 substantiate this notion insofar as they demonstrate a parallel in the exudate-seeking capacity of labeled S-phase lymphocytes and the ability of "immune" TDL to protect recipient rats against an i.v. *Listeria* challenge. In each case, inhibition by I-NDV was proportional to the concentration of virus in the incubation mixture.

Resistance of Listeria-Immune Lymphocytes to Inhibition by Passively Trans-

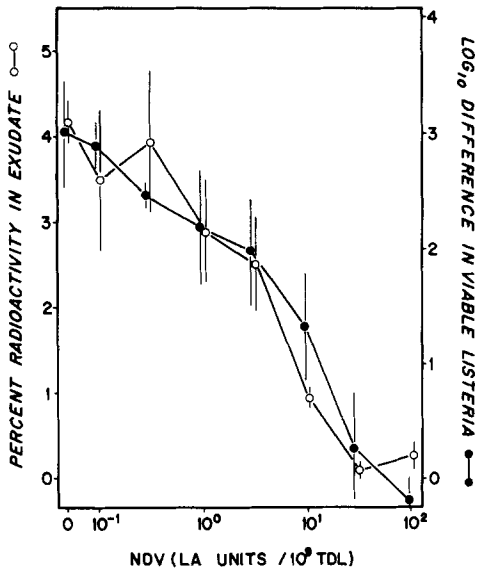

FIG. 2. Curves showing the effect of various concentrations of I-NDV on the exudate-seeking capacity of labeled immunoblasts and the ability of *Listeria*-immune TDL to protect recipient rats against a *Listeria* challenge. Radioactivity in exudates harvested 24 h after cell transfer (○—○) is expressed as percent counts per minute originally present in the donor inoculum. The protective activity of the donor cells (●—●) is expressed as the difference in viable organisms in the spleens of individual cell recipients and the average bacterial burden in a panel of nonimmunized controls sacrificed 48 h after i.v. challenge with 3.64×10^6 *L. monocytogenes*. Means of 5 ± SD.

ferred Virus. In the foregoing experiment, a small but unavoidable number of infectious virions were carried into rats at the time of cell transfer. Therefore, the question arises whether passenger virus can interact with the recipient's own lymphocytes or macrophages in a manner that prevents full expression of cellular resistance to infection. The problem was studied by incubating *Listeria*-immune TDL in vitro with I-NDV, 50 LA units/10^9 cells. The TDL were then washed and added to an equal number of untreated lymphocytes from either the same *Listeria*-immune rats or from normal donors. It was reasoned that passenger virus might interact with other potentially vulnerable lymphocytes thereby abrogating their capacity to immunize adoptively.

But this was not the case. Table I indicates that cell mixtures containing treated and untreated "immune" TDL conferred a full measure of immunity on the liver and only a modestly low level of resistance on the spleen. Failure of passively transferred virus to inhibit untreated lymphocytes in the same inoculum makes it unlikely that it would compromise the immunological performance of the recipient's own cells.

Effect of I-NDV on the Mediator of DTH. TDL taken from rats at the peak of their reponse to an immunizing infection with *L. monocytogenes* can confer DTH to LMA on normal recipients (13). The cells which do so are generated in response to infection and are thought to be linearly related if not identical with those that immunize adoptively (20). Therefore, it was pertinent to inquire

TABLE I
Failure of Passenger Virus to Inhibit the Protective Capacity of Listeria-Immune TDL

TDL inoculum*	Log_{10} protection‡	
	Liver	Spleen
Immune (untreated) + normal (untreated)	2.03	2.80
Immune (untreated) + immune (I-NDV)	2.02	2.09
Normal (untreated) + immune (I-NDV)	−0.31	−0.12

* Obtained from 6-day *Listeria*-immune donors. Cells incubated in vitro in either virus-free medium (untreated) or medium containing 50 LA units of I-NDV/10^9 cells. The TDL were then combined in equal numbers with one another or with untreated TDL from normal, nonimmunized rats. Cell mixtures containing 4×10^6 TDL/g of body weight were infused into syngeneic recipients 1 h after the latter had been challenged i.v. with 3.27×10^6 *L. monocytogenes*.

‡ Mean difference 48 h after challenge in viable *Listeria* in the tissues of five adoptively immunized subjects and five nonimmunized controls.

TABLE II
Inhibition by I-NDV of Specifically Sensitized Lymphocytes which Transfer DTH to LMA

Test subjects	DTH index*
Donors of *Listeria*-immune TDL	4.67 ± 1.65
Recipients of 2×10^8 untreated TDL	2.17 ± 0.31
Recipients of 2×10^8 NDV-treated TDL‡	1.10 ± 0.19

* Means of 5 ± SD.

‡ Incubated in vitro with 50 LA units of I-NDV/10^9 cells.

whether the cells responsible for transferring DTH can be inhibited by I-NDV. Table II shows that an i.v. injection of 2×10^8 *Listeria*-immune TDL conferred a low but significant ($P < 0.01$) level of DTH on normal recipients, and that their ability to do so was abrogated by brief exposure to NDV in vitro.

Failure of I-NDV to Inhibit Antigen-Sensitive T Cells. Antigen-sensitive T cells of the kind which initiate graft-vs.-host reactions are more resistant than activated T cells to the inhibitory influence of NDV. This conclusion was drawn from experiments in which the proliferative capacity of Lewis TDL was measured in the spleens of heavily irradiated (Lewis × DA)F_1 hybrid recipients.

The results of a typical experiment are shown in Table III where it can be seen that treatment of the donor cells with I-NDV had little if any effect upon the level of [^{3}H]thymidine incorporation in the spleen. Since the recipients had been irradiated (850 rads), it seems likely that the proliferating cells were mainly antigen-sensitive lymphocytes originally present in the donor inoculum (15). It is in keeping with this notion that a low level of radioactivity was detected in the spleens of uninoculated F_1 rats and rats given TDL which had been incubated in vitro with mitomycin-C.

Effect of UV-NDV on Listeria-Immune Lymphocytes. It was of interest to determine whether the inhibitory influence of I-NDV on the protective function of Listeria-immune lymphocytes is related, in part, to the ability of the virus to replicate in susceptible cells. To test this proposition, immune TDL were incubated in vitro with I-NDV or UV-NDV. The TDL were then washed and infused

TABLE III
*Failure of I-NDV to Inhibit the Proliferative Capacity of Antigen-Sensitive Lymphocytes**

Treatment of donor TDL	No. lymphocytes transferred × 10^{-3}/g	Spleen cpm × 10^{-3}‡
NDV§	100	492 ± 112
	10	230 ± 45
None‖	100	617 ± 151
	10	230 ± 47
	0	19 ± 24
Mitomycin-C¶	100	50 ± 31

* TDL from normal Lewis rats were infused into irradiated (850 rads) (Lewis × DA)F_1 hybrid recipients.
‡ Cell-associated radioactivity in the spleen 4 days after cell transfer. The animals were sacrificed 1 h after an i.v. pulse of 1 μCi/g of [^{3}H]thymidine. Means of 5 ± SD.
§ Incubated in vitro with 50 LA units of I-NDV/10^9 cells.
‖ Incubated in virus-free medium.
¶ Incubated in medium containing 50 μg/ml of mitomycin-C.

TABLE IV
Effect of UV Irradiation on the Capacity of NDV to Inhibit the Protective Function of Listeria-Immune TDL

Treatment of donor TDL*	NDV (EID_{50}/ml)‡	Log_{10} protection§	
		Liver	Spleen
I-NDV	$10^{10.4}$	−0.44	−0.52
UV-NDV‖	$<10^{1.0}$	2.59	1.44
None		2.70	2.14

* Cells from 6-day *Listeria*-immune donors were incubated in vitro in either virus-free medium (none) or medium containing 50 LA units of NDV/10^9 cells.
‡ Infectivity measured in 10-day embryonated eggs.
§ Mean difference 48 h after challenge in viable *Listeria* in the tissues of five adoptively immunized subjects and five nonimmunized controls.
‖ Irradiated (2.58×10^4 ergs/mm^2) immediately before addition to the culture.

into normal recipients immediately after the latter had been challenged i.v. with 3.42×10^6 *L. monocytogenes*.

The results are shown in Table IV where it can be seen that I-NDV abrogated the protective power of the transferred cells. By comparison, UV-NDV had only a modest inhibitory influence on the level of adoptive immunity conferred on the spleen and none whatsoever on resistance in the liver.

Effect of UV-NDV on Lymphocyte Traffic. A double-labeling technique was used to mark cohorts of dividing and nondividing lymphocytes in the thoracic duct lymph of donor rats. The prospective donors were given 14 daily injections of [^{3}H]thymidine (0.5 μCi/g). 9 days later, the animals were infected with *L. monocytogenes*. All were incannulated on the 5th day of infection. Cells issuing from the fistulae were collected, pooled, and incubated in vitro with

[^{14}C]thymidine. Autoradiographic analysis revealed that 97.7% of the cell-associated ^{3}H was vested in small lymphocytes, whilst the ^{14}C was concentrated in heavily labeled immunoblasts. Cells doubly labeled in this manner were incubated in virus-free medium or in medium containing either I-NDV or UV-NDV. They were then washed and 2×10^8 were infused into each of 90 *Listeria*-infected recipients. The cells were transferred immediately after the recipients had been stimulated i.p. with killed *L. monocytogenes*.

Fig. 3 indicates that treatment with NDV had only a modest inhibitory effect upon the accumulation of ^{3}H-labeled small lymphocytes in lymph nodes. Inhibition was observed after treatment with either I-NDV or UV-NDV and was most conspicuous during the first 6 h after cell transfer.

But the two virus preparations had entirely different effects upon the localization in exudates of ^{14}C-labeled immunoblasts. Whereas I-NDV virtually abolished the exudate-seeking capacity of labeled immunoblasts, treatment with UV-NDV merely impeded their influx during the first few hours after transfer (Fig. 4). In some experiments (results not shown) treatment with UV-NDV failed to influence the final composition of the exudate, as judged by the level of radioactivity found at 24 h. These findings encourage the belief that UV-NDV has but a transient influence on immunoblast traffic, similar to the effect of virus on the revivescent homing of small lymphocytes to lymph nodes.

Effect of Deferred Stimulation on Localization of Labeled Immunoblasts in Induced Peritoneal Exudates. In an earlier study (21) it was shown that labeled S-phase TDL move in substantial numbers from the blood into *Listeria*-induced exudates only during the early postinduction period. This raises the fascinating possibility that immunoblasts recover fully from changes induced by NDV but fail to localize in exudates because local changes in the microcirculation no longer favor their extravasation. To test this proposition, a TDL suspension containing labeled immunoblasts was exposed briefly to I-NDV or UV-NDV before infusion into recipient rats. The latter were stimulated i.p. either at the time of cell transfer or 6, 12, or 24 h after transfer. It was reasoned that if immunoblasts recover from the effects of virus treatment, they would localize more efficiently in exudates induced several hours after transfer.

The results in Fig. 5 could be interpreted in this way, at least insofar as treatment with UV-NDV is concerned. A small but significant ($P < 0.05$) reduction in radioactivity was observed in exudates borne by rats given UV-NDV-treated TDL, but only when the animals were stimulated i.p. at the time of cell transfer. When stimulation was deferred, similar levels of radioactivity were found in animals given treated or untreated lymphocytes. In contrast, treatment with I-NDV curtailed the influx of labeled immunoblasts into exudates regardless of the interval between cell transfer and stimulation. These findings give credence to the notion that NDV has at least two effects on S-phase lymphocytes. One effect is related in some way to virus adsorption and is mediated by I-NDV or UV-NDV. The other effect is irreversible and is mediated by infectious virus only.

The Blocking Activity of NANA. NDV is one of several viruses which can bind to rat TDL (22). Adsorption is readily demonstrated at 4°C. But the virus-cell interaction is unstable; upon warming to 37°C virus elutes, sialic acid is

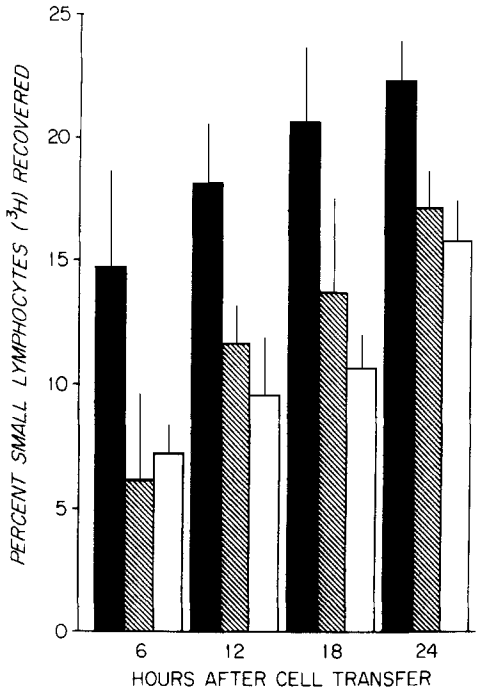

FIG. 3. Cell-associated radioactivity in the visceral lymph nodes of *Listeria*-infected rats. The animals had been infused with a population of doubly labeled TDL from similarly infected donors (see text). Treatment of the donor cells in vitro with I-NDV (□) or UV-NDV (▨) impeded the homing of [^{3}H]thymidine-labeled small lymphocytes, but only during the first 6 h after transfer. Thereafter virus-treated cells accumulated in the nodes at approximately the same rate as similarly labeled but otherwise untreated lymphocytes (■). Means of 5 ± SD.

released, and the cells cannot be agglutinated again by NDV (23). These events have been ascribed to destruction of specific membrane receptors by viral neuraminidase, and have been taken as evidence that the receptors terminate in sialic acid. It is in keeping with this hypothesis that NANA can block binding of NDV to rat TDL (22). Since the inhibitory influence of I-NDV is dependent upon its adsorption to susceptible cells, it is logical to postulate that virus-induced changes in T-lymphocyte function would themselves be inhibited by NANA.

The results in Table V substantiate this notion insofar as the exudate-seeking capacity of labeled S-phase lymphocytes is concerned. Likewise, Table VI shows that NANA can impede the interaction of I-NDV with *Listeria*-immune TDL.

The blocking effect of NANA is specific because other saccharides do not inhibit at the same molar concentration. Table VII indicates that of the various sugars and aminosugars added to the culture medium, NANA alone obstructed the interaction of I-NDV and *Listeria*-immune TDL.

Blocking of I-NDV by UV-NDV. Since UV-NDV can destroy membrane receptors for the virus, it was pertinent to inquire whether such receptor-depleted lymphocytes enjoy a measure of resistance against I-NDV. This possibility was tested by incubating *Listeria*-immune TDL in vitro with UV-NDV.

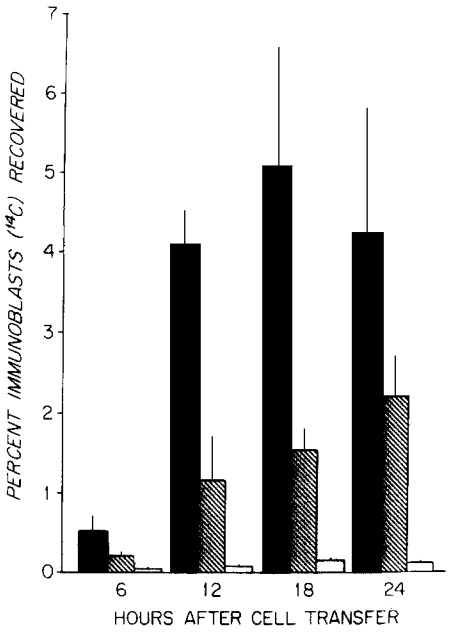

FIG. 4. Cell-associated radioactivity in peritoneal exudates borne by the same groups of rats shown in Fig. 3. [^{14}C]thymidine-labeled immunoblasts originally present in the donor inoculum localized in substantial numbers in the exudates (■). Treatment of the donor cells with I-NDV (□) virtually abolished their exudate-seeking capacity, whereas UV-NDV (▨) had only a modest inhibitory effect that was most conspicuous during the first 6–12 h after transfer. Means of 5 ± SD.

The TDL were then washed and resuspended in fresh virus-free medium or medium containing I-NDV. After incubation for an additional 60 min at 37°C, the cells were tested for their capacity to protect recipient rats against an i.v. *Listeria* challenge.

The results are summarized in Table VIII where it can be seen that UV-NDV had a modest inhibitory influence upon the level of immunity conveyed by transferred cells. But TDL, treated initially with UV-NDV, were resistant to further inhibition by a concentration of I-NDV that erased the protective power of untreated lymphocytes. The results could be interpreted in several ways; however, they accord with the view that irradiated virus can cleave specific membrane receptors thereby preventing adsorption of I-NDV and replication of the virus in activated T cells (7).

Discussion

NDV is one of the several viruses which can adsorb to lymphocytes and transiently interrupt their recirculation through lymph nodes and spleen (4–6). The phenomenon, first demonstrated by the Woodruffs (6) in experiments using ^{51}Cr-labeled TDL, was confirmed in the current study using either [5-^{3}H]uridine or [^{3}H]thymidine to selectively label small lymphocytes. Cells collected from the thoracic duct lymph of *Listeria*-infected rats were exposed to NDV in vitro. After treatment with virus and infusion into recipient rats, labeled small lymphocytes were transiently deflected from lymph nodes.

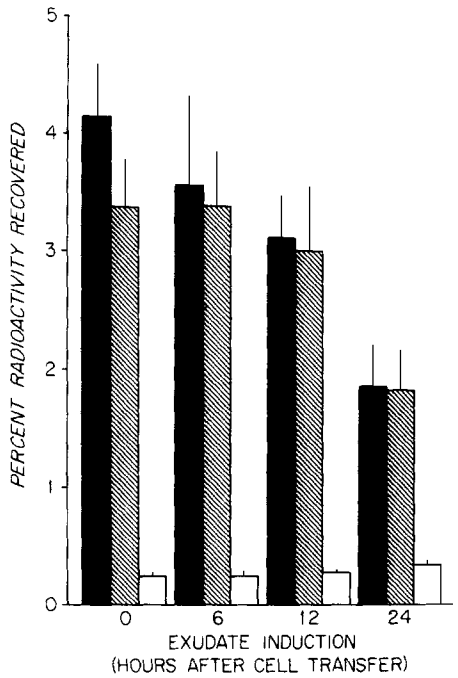

FIG. 5. Cell-associated radioactivity in peritoneal exudates borne by *Listeria*-infected rats which had been infused with [^{14}C]thymidine-labeled immunoblasts from the thoracic duct lymph of similarly infected donors. The exudates, induced at various intervals with respect to cell transfer, were harvested 24 h after stimulation. Treatment of the donor cells with I-NDV (□) inhibited their exudate-seeking capacity regardless of the interval between cell transfer and stimulation. By comparison, treatment with UV-NDV (▨) had only a modest inhibitory effect, and then only when exudates were induced at the time of cell transfer. The solid bars indicate the accumulation in exudates of similarly labeled but otherwise untreated immunoblasts. Means of 5 ± SD.

TABLE V

*Blocking by NANA of the Interaction of I-NDV and Exudate-Seeking Immunoblasts**

Concentration of NANA in culture (pM/LA unit)	Percent radioactivity in exudate‡
0	0.11 ± 0.03
10^0	0.15 ± 0.06
10^1	0.29 ± 0.13
10^2	0.82 ± 0.09
10^3	1.77 ± 0.17
0§	3.74 ± 0.50
10^3‖	3.95 ± 0.83

* Obtained from the thoracic duct lymph of 6-day *Listeria*-immune donors. Cells incubated in vitro with I-NDV were infused into similarly infected recipients. The latter were stimulated i.p. at the time of cell transfer.

‡ Harvested 24 h after stimulation. Means of 5 ± SD.

§ Incubated in virus-free medium.

‖ Incubated in medium containing NANA but no virus.

TABLE VI
*Blocking by NANA of the Interaction of I-NDV and Listeria-Immune TDL**

Concentration of NANA in culture (pM/LA unit)	Log_{10} protection (spleen)‡
0	0.10
10^0	−0.35
10^1	−0.16
10^2	1.25
10^3	2.35
0§	3.33
$10^{3\|}$	3.60

* Obtained from 6-day *Listeria*-immune donors. Cells incubated in vitro with I-NDV were infused into normal recipients 1 h after the recipients had been challenged i.v. with 2.50×10^6 *L. monocytogenes*.
‡ Mean difference 48 h after challenge in viable *Listeria* in the spleens of five adoptively immunized subjects and five nonimmunized controls.
§ Incubated in virus-free medium.
‖ Incubated in medium containing NANA but no virus.

TABLE VII
*Effects of Various Sugars on the Interaction of I-NDV and Listeria-Immune TDL**

Sugar (50 μM)	Log_{10} protection (spleen)‡
L-fucose	−0.20
D-mannose	0.02
D-galactose	0.06
N-acetyl-galactosamine	−0.06
N-acetyl-glucosamine	−0.19
N-acetyl-neuraminic acid	2.57
None	0.11
None§	3.36

* Cells and I-NDV (50 LA units/10^9 TDL) were incubated in vitro in medium containing various sugars. The cells were then washed and infused into normal recipients 1 h after the recipients had been challenged i.v. with 2.85×10^6 *L. monocytogenes*.
‡ Mean difference 48 h after challenge in viable *Listeria* in the spleens of five adoptively immunized subjects and five nonimmunized controls.
§ Incubated in virus-free medium.

The class(es) of small lymphocytes affected by NDV was not determined; however, there are reasons for thinking that virus-treated T cells are diverted from lymphoid tissue to the liver (4). It is in keeping with this view that i.v. injection of NDV into rats or mice causes rapid contraction of the circulating lymphocyte pool and depletion of small lymphocytes in areas of lymphoid tissue normally occupied by T cells (6). UV-NDV and I-NDV are active in this regard. Furthermore, both can block localization of labeled small lymphocytes in lymph

TABLE VIII
*Failure of I-NDV to Inhibit Listeria-Immune TDL after Treatment of the Cells with UV-NDV**

Treatment of donor TDL		Log_{10} protection‡	
First incubation	Second incubation	Liver	Spleen
UV-NDV	None	1.33	1.27
UV-NDV	I-NDV	1.61	1.88
None	I-NDV	−0.59	−0.39
None	None	1.89	2.27

* TDL from 6-day *Listeria*-immune donors were incubated serially in virus-free medium (none) or medium containing either UV-NDV or I-NDV. Cells treated in this manner were infused into normal recipients 1 h after the recipients had been challenged i.v. with 2.83×10^6 *L. monocytogenes*.

‡ Mean difference 48 h after challenge in number of viable *Listeria* in the tissues of five adoptively immunized subjects and five nonimmunized controls.

nodes. The ability of UV-NDV to inhibit T-cell homing indicates that the migratory behavior of the cells does not depend upon the ability of the virus to achieve a complete replication cycle in infected cells.

A novel aspect of this investigation was the demonstration that NDV can interact with several classes of T cells as defined by their cytokinetics, tissue disposition, and immunological performance. Newly formed T cells, particularly those in active cycle, are especially vulnerable. Cells of this type are delivered in increased numbers to the thoracic duct lymph of rats infected with *L. monocytogenes* (14). The cells have a low density of surface immunoglobulin (19), a penchant to localize in inflammatory foci (21), and numbered among them are lymphocytes which can protect recipient rats against an i.v. *Listeria* challenge. In experiments using I-NDV, a precise and quantitative relationship was found between the ability of the virus to impede the exudate-seeking capacity of labeled S-phase lymphocytes and their protective power in adoptively immunized subjects (Fig. 2).

The interaction of NDV with rat T cells is dependent upon attachment of the virus to specific receptors on the plasma membrane. These receptors either terminate in sialic acid or are so positioned that sialic acid residues favor attachment of the virus. Evidence to support this notion was obtained by the Woodruffs (22). They demonstrated that agglutination of TDL by NDV is inhibited in medium containing NANA or fetuin, a sialoglycoprotein. In the present investigation, it was shown that both exudate-seeking S-phase lymphocytes and *Listeria*-immune TDL can be insulated by NANA against the inhibitory influence of infectious virus. These observations, taken in conjunction with the Woodruffs' findings (22), suggest that receptors for NDV are expressed on T cells regardless of size, state of activation, or their position in the mitotic cycle.

Several lines of evidence indicate that NDV has at least two effects on T cells. One effect is related in some way to virus adsorption and is revealed in transient displacement of the cells from their usual traffic zones in the body. Both dividing and nondividing lymphocytes are affected. The influence on T-cell migration is reversible and is observed after treatment with either I-NDV or UV-NDV. The

second effect is irreversible and is mediated by infectious virus only; but the mechanism has not been determined. Nevertheless, it is logical to postulate that I-NDV has a lethal effect on activated T cells, because they alone support productive growth of the virus (7). Interesting in this connection was the finding that UV-NDV can protect *Listeria*-immune TDL against the inhibitory influence of infectious virus. This seeming paradox could be explained if irradiated virus can destroy specific receptors on activated T cells, thereby preventing their infection and destruction by I-NDV.

In any case, it is evident that T cells are not equally vulnerable. I-NDV can readily abrogate the protective power of *Listeria*-committed T cells. By comparison, even high concentrations of virus have little if any impact upon proliferation in the spleen of histocompatibility antigen-reactive T cells (Table III). The reasons for this are not far to seek when it is remembered that the cells concerned are recirculating small lymphocytes, and that treatment with virus merely deflects them from the spleen during the first few hours after i.v. transfer (4).

Activated T cells do not belong to a single, homogeneous class (18, 24–26). Therefore the question arises whether the cells inhibited by I-NDV belong to a particular subset as defined by morphological, cytokinetic, or functional criteria. The problem has been studied by Bloom and his associates in experiments using vesicular stomatitis virus (VSV). VSV can replicate in activated lymphocytes and inhibit T-cell function in vitro and in vivo (Nowakowski, Kano, Bloom, Romano and Thorbecke, unpublished results). The cells supporting virus growth have been identified morphologically as "large lymphocytes" (27), and functionally as T cells on the basis of their response to mitogens, inhibition by anti-Thy 1 serum, and failure to develop in cultures containing spleen cells from nude (athymic) mice (7, 28). An increase with time in virus plaque-forming cells was observed in cultures containing purified protein derivative of tuberculin and peripheral blood lymphocytes from tuberculin-sensitive human donors (29). The increase was linear and was unaffected by addition of vinblastine to the culture medium. This, and other evidence derived from studies of mixed lymphocyte reactions (28), point to a nondividing T cell as the principal target of virus-induced suppression.

NDV also can replicate in activated lymphocytes (30). However, the permissive cells have not been identified, nor has it been formally demonstrated that the immunosuppressive influence of I-NDV is related solely to virus growth and lysis of infected cells. The fact that *Listeria*-immune T cells are especially vulnerable to NDV is interesting in this connection, because the cells are newly formed and are inhibited by vinblastine (9). I-NDV also can abrogate the protective power of TDL taken from rats infected with BCG; but suppression by virus is observed only when the cells are collected at an early stage of immunization and are in active cycle (McGregor, unpublished results).

How can these findings be reconciled with the known capacity of VSV to replicate in nondividing lymphocytes (28, 29)? One possibility is that NDV and VSV inhibit different subsets of activated T cells. This can be proposed without prejudice to the debate concerning the lineal relationship of T-helper cells, cytotoxic T cells, and specifically sensitized lymphocytes which collaborate with

macrophages in the expression of DTH and cellular resistance to infection. Alternatively, NDV and VSV might inhibit activated T cells regardless of their functional commitment, provided the appropriate conditions for virus replication are satisfied. According to this hypothesis, long-lived T cells become permissive only when they are "stimulated" in vitro by specific antigens or a polyclonal mitogen. Further stimulation may not be necessary when the cells are newly formed or in active cycle. This is the case in *Listeria*-infected rats, in animals recently infected with BCG, and during the early phase of a graft-vs.-host reaction (Nowakowski, Kano, Bloom, Romano, and Thorbecke, unpublished results). In any case, the ability of viruses to inhibit activated T cells has implications with respect to the host's defense against infection, virus-induced immunosuppression, and possibly certain immunopathological sequelae of viral disease (3, 31).

Summary

Newcastle disease virus (NDV) can interact in at least two ways with rat T cells. By adsorbing to circulating lymphocytes, the virus can transiently deflect the cells from lymph nodes and inflammatory exudates induced in the peritoneal cavity. T cells are affected regardless of age, state of activation, or position in the mitotic cycle. The effect is reversible and is mediated not only by infectious (I)-NDV, but also by UV-NDV which cannot achieve a complete replication cycle in eggs. But I-NDV has another lasting effect on activated T cells. It is revealed in the failure of virus-treated thoracic duct lymphocytes to transfer cellular resistance to *Listeria monocytogenes*, delayed-type hypersensitivity to soluble antigens of the parasite, and the permanent exclusion of labeled S-phase lymphocytes from inflammatory foci. Activated T cells are inhibited by virus multiplicities which have little if any effect upon the proliferative potential of antigen-sensitive T cells or localization of labeled small lymphocytes in lymph nodes. The underlying mechanism has not been determined; however, there are reasons for thinking that NDV has a lethal effect upon activated T cells, because the latter are permissive for virus replication.

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