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Lymphocyte Populations in Acute Viral Gastroenteritis

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Viral gastroenteritis was induced in 16 of 24 normal volunteers after oral administration of either the Norwalk or Hawaii agents. Clinical illness lasted for 24 to 48 h and resolved spontaneously. During acute illness, a transient lymphopenia was noted which involved all lymphocyte subpopulations (thymus- and bone marrow-derived, and null cells). No circulating lymphocytotoxins were detected, and the lymphocytes remaining in the circulation responded normally to mitogenic stimuli. The acute lymphopenia occurred at the time that mononuclear cell infiltration of the jejunal mucosa has been noted. These findings are consistent with the occurrence of a redistribution of circulating lymphocytes during acute illness, with accumulation of lymphocytes at the site of infection in the gut.

Viral infections have been reported to have varied effects upon the numbers, distribution, and functions of circulating lymphocytes (3, 15, 41). Viral infections of the gastrointestinal tract appear to be among the most common illnesses experienced by American families (6). As part of studies on the etiology and pathogenesis of viral gastroenteritis in man, we undertook the sequential examination of circulating lymphocyte populations during this common illness, which was experimentally induced in normal volunteers by the administration of the Norwalk (8) or Hawaii (42) agent of viral gastroenteritis. These two recently described, antigenically distinct (42), parvovirus-like (8, 25, 42) agents induce a self-limited syndrome in both adults and children, which lasts for 24 to 48 h and consists of vomiting and/or diarrhea, often accompanied by low-grade fever, abdominal cramps, and malaise. Peripheral venous blood samples were obtained before, 48 h after, and 2 weeks after inoculation. These samples were analyzed for total lymphocyte and monocyte counts, proportions of lymphocyte subpopulations, and the in vitro lymphocyte blastogenic response to stimulation by mitogens and viral antigens.

MATERIALS AND METHODS

Volunteer studies. Experimental subjects were 24 normal volunteers, aged 18 to 25, at the Clinical Center of the National Institutes of Health. Studies were carried out with informed consent and under close clinical supervision as previously described (7).

Inocula. The inocula containing either the Norwalk or Hawaii agent were 2% stool filtrates diluted in veal infusion broth (7) and were free of bacterial, fungal, parasitic, or other viral agents as previously described (27). After an overnight fast, volunteers ingested 2 g of $NaHCO_3$. Five minutes later, they drank 5 ml of the filtrate diluted in 30 ml of water.

Volunteers who developed documented vomiting and/or diarrhea (unformed stool) along with symptoms such as malaise, cramps, or anorexia were considered to have definite illness.

Total lymphocyte and monocyte counts. Leukocyte counts were performed with a Coulter counter (model Fn, Coulter Electronic, Inc., Hialeah, Fla.), and differential counts were performed on peripheral blood smears stained with Wright's stain. Two hundred cells were counted per smear.

Preparation of lymphocyte suspensions. Mononuclear cells were obtained by Hypaque-Ficoll separation of heparinized blood obtained before, 48 h after, and 2 weeks after inoculation of the volunteers with the viral agents. Mononuclear cells were washed three times in Eagle minimal essential media modified for suspension culture (MEM-S).

Determination of proportion of T- and B-lymphocytes. Thymus-derived (T) cells were determined by the spontaneous sheep erythrocyte (SRBC) rosette method as previously described (22). Briefly, 0.25 ml of the lymphocyte suspension containing 4×10^6 lymphocytes/ml in MEM-S was added to 0.25 ml of 0.5% SRBC in Hanks balanced salt solution, incubated at 37°C for 5 min, and then centrifuged at 200 $\times$ *g* for 5 min. The supernatant was aspirated, and 0.5 ml of fetal calf serum absorbed with SRBC was added. The cells were centrifuged at 200 $\times$ *g* for 5 min at 4°C and incubated overnight at 4°C. One-third of the supernatant was discarded, and the cells were gently resuspended. A drop of the suspension was placed under a cover slip on a slide, and 400 cells were counted under phase-contrast microscopy. Lymphocytes binding three or more SRBC were counted as rosette-forming T cells.

Bone marrow-derived (B) cells were determined by the complement receptor assay (5). One milliliter

containing 10% SRBC (E) previously sensitized with rabbit 19S anti-SRBC stroma antibody (A) was incubated with 1 ml of 1:10 dilution of mouse serum in isogiver's solution as a source of complement (C). The resulting EAC was incubated at 37°C for 45 min, washed three times with 0.01 M ethylenediaminetetraacetate, and reconstituted with 5 ml 0.01 M ethylenediaminetetraacetate. A 0.25-ml amount of this mixture was added to 0.25 ml of the lymphocyte suspension containing 4×10^6 cells/ml and incubated on a rotator at 37°C for 45 min. A drop of the suspension was placed under a cover slip, and 400 cells were counted under phase-contrast microscopy. Lymphocytes were distinguished from monocytes by morphology and phagocytosis of latex particles. Lymphocytes that bound three or more sheep cells were counted as EAC rosette-forming B cells. B cell determinations by this method correlated closely with determination by the presence of surface immunoglobulins, when lymphocytes from normal populations were examined in our laboratory and elsewhere (23).

Lymphocyte cultures. Quadruplicate cultures containing 1×10^5 cells in 0.2 ml of MEM-S supplemented with 0.02 M L-glutamine, 100 U of penicillin per ml, 100 μg of streptomycin sulfate per ml, and 15% homologous AB serum were established in microtiter plates (Cooke Laboratory Products, Alexandria, Va.). Varying concentrations of phytohemagglutinin (PHA) (Wellcome Reagents, Ltd., Beckenham, England), concanavalin A (Con A) (Nutritional Biochemicals, Corp., Cleveland, Ohio), or Norwalk or Hawaii filtrates were added to the cultures, which were then incubated at 37°C in 5% CO_2 at 100% humidity. Three days after the addition of mitogens or 5 days after the addition of virus-containing filtrates, 0.2 μCi of tritiated thymidine (New England Nuclear, Boston, Mass.) was added to each culture. After 4 h, the cells were collected on fiberglass filters, washed with 10% trichloroacetic acid and 95% ethanol, and placed in 10 ml of Aquasol (New England Nuclear) (16). The trichloroacetic acid precipitable activity was counted in a liquid scintillation counter, model LS-350 (Beckman Instruments, Inc., Fullerton, Calif.). The arithmetic mean of quadruplicate cultures was determined, and the degree of stimulation was expressed as the difference in counts per minute per 10^6 lymphocytes between stimulated and unstimulated (control) cultures.

Lymphocytotoxic antibodies. Examination of sera for the presence of lymphocytotoxic antibodies against lymphocytes from 100 individual donors was performed by P. Terasaki by the microdroplet assay previously described (39).

Cortisol levels. Plasma samples (8 a.m.), obtained at the time that lymphocyte studies were performed, were assayed for cortisol concentrations by the methods of Van der Vies (40).

RESULTS

Clinical illness. The clinical characteristics of illness induced by Norwalk and Hawaii agents have been described in detail previously (7, 42), and are summarized in Table 1. In the current study, 9 of 16 volunteers who received the Norwalk inoculum manifested definite illness. Seven had both vomiting and diarrhea, one had vomiting alone, and one had diarrhea alone. Seven of nine ill volunteers had low-grade fever (>99.4°F orally) (ca. 37.4°C). Mean incubation period for illness was 31 h, and illness lasted for 24 to 48 h. Seven of eight volunteers who received the Hawaii inoculum manifested definite illness. Four had both vomiting and diarrhea, two had diarrhea alone, and one had vomiting alone. Four had low-grade fever. Mean incubation period for illness was 33 h, and disease manifestations lasted for 24 to 48 h. Illness induced by the Norwalk agent was clinically indistinguishable from that induced by the Hawaii agent. All volunteers recovered spontaneously, completely, and without sequelae. The eight volunteers who were described as "not ill" remained asymptomatic during the study, without fever, vomiting, or diarrhea.

Peripheral lymphocyte and monocyte counts. Lymphopenia was noted 48 h after inoculation in eight of nine volunteers with Norwalk-induced disease (Fig. 1). Compared to mean (± standard error of the mean) preinoculation lymphocyte counts of 2,150 (±434)/mm³, lymphocyte counts at 24 h rose to 2,661 (±293) (not significant [NS], $P > 0.05$, Student's t test), but fell to 734 (±130) ($P < 0.01$) at 48 h. They then rose to 1,573 (±269) at 72 h, and returned to base-line levels, 2,091 (±305) by 6 days after inoculation.

Similarly, in Hawaii-induced disease, lymphopenia occurred in six of seven patients 48 h

TABLE 1. *Clinical characteristics of gastroenteritis induced by Norwalk and Hawaii agents*

Agent of viral gastroenteritis	No. of volunteers	No. ill	No. with indicated finding			Incubation period	Duration of illness (h)
			Fever[a]	Vomiting	Diarrhea		
Norwalk	16	9	7	8	8	31 (18–36)[b]	24–48
Hawaii	8	7	4	5	6	33 (18–37)	24–48

[a] >99.4°F orally (ca. 37.4°C).
[b] Range.

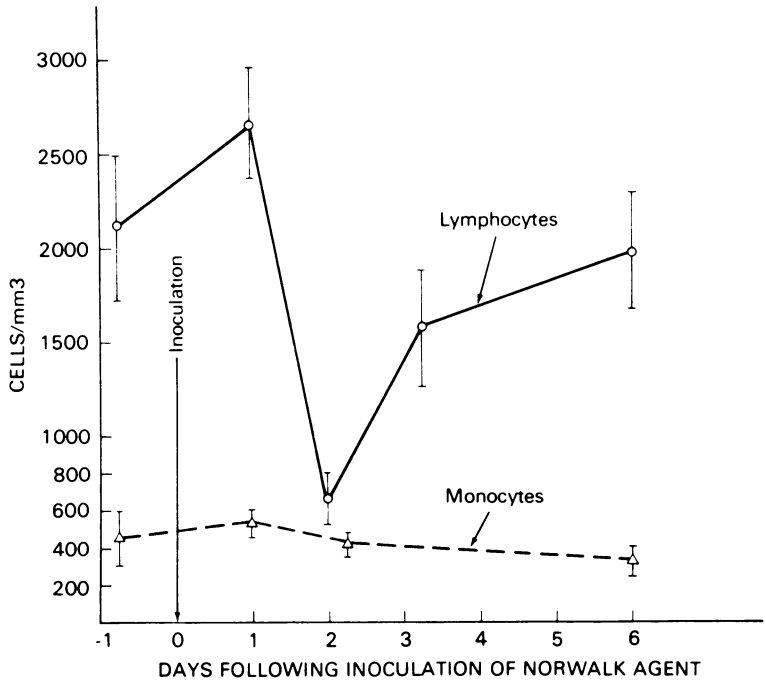

FIG. 1. *Circulating lymphocyte and monocyte counts in volunteers with Norwalk agent-induced gastroenteritis. Norwalk agent was orally administered at day 0. Values represent mean ± SEM counts at various time points after inoculation.*

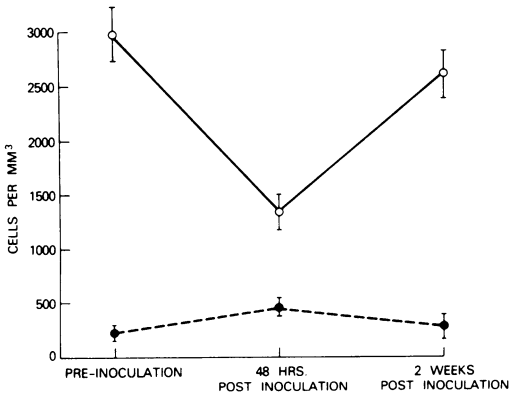

FIG. 2. *Circulating lymphocyte and monocyte counts in volunteers with Hawaii agent-induced gastroenteritis. Values represent mean ± SEM counts at various time points following inoculation. Symbols: ○, lymphocyte count; ●, monocyte count.*

after inoculation, from a base-line level of 2,975 (±256) to 1,333 (±176) ($P < 0.001$) (Fig. 2). Two weeks postinoculation, lymphocyte counts had returned to normal.

Monocyte counts remained stable throughout Norwalk-induced disease (Fig. 1), but rose slightly from 213 (±55)/mm³ to 455 (±85)/mm³ ($P < 0.05$) in the sample obtained 48 h after inoculation with the Hawaii agent (Fig. 2).

T and B lymphocyte populations. Absolute numbers of both T and B cells fell by 48 h postinoculation in Hawaii-induced disease (Fig. 3). T cells fell from preinoculation values of 1,920 (±235)/mm³ to 778 (±210) ($P < 0.05$) and returned to 1,980 (±176) by 2 weeks postinoculation. B cells fell from 341 (±26) to 191 (±22) ($P < 0.05$) and returned to 448 (±70) by 2 weeks after inoculation. The mean percentage decrease of T cells at 48 h (58.15 ± 10.4) was not significantly different ($P > 0.05$) from the decrease in B cells (40.59 ± 8.9) at that time point. The percentage of the total lymphocyte count made up of T cells fell from 64 to 52% (NS) at 48 h and subsequently returned to 74%. The percentage of B cells remained relatively constant (12%, 15%, 17%) (NS) throughout the study.

The absolute numbers of null cells (calculated by substracting the sum of T and B cells from the total lymphocyte count) similarly fell at 48 h postinoculation (Fig. 3), and remained at that level at 2 weeks postinoculation, since both T and B cells had risen slightly above their preinoculation levels in the 2 weeks postinoculation sample.

T and B lymphocyte populations were exam-

ined in two volunteers with Norwalk-induced illness. T lymphocytes fell from preinoculation values of 1,417 (±62) to 506 (±67) at 48 h, B cells fell from 312 (±70) to 176 (±12), and null cells from 606 (±72) to 303 (±54). Two weeks postinoculation, T and B cells had returned to preinoculation levels. T and B cell decreases were not statistically significantly different ($P > 0.05$) from those seen in Hawaii-induced illness.

Eight volunteers who received either the Norwalk or Hawaii agent and remained asymptomatic throughout the study manifested no significant changes in total peripheral lymphocyte counts or in circulating lymphocyte subpopulations.

Mitogen stimulation of peripheral lymphocyte cultures. Lymphocyte cultures obtained from volunteers with Hawaii-induced disease were stimulated with PHA over a dose range of 0.25 to 5.0 μg/ml (Fig. 4) and Con A over a range of 1.0 to 50.0 μg/ml (Fig. 5). Cultures taken before, 48 h after, and 2 weeks after inoculation had similar responses, though the counts at the peak-response doses of the mitogens were slightly higher in the 48-h postinoculation sample, compared with the pre- and 2-week postinoculation samples for both PHA ($P < 0.01$) and Con A ($P < 0.05$). Mean counts per minute in unstimulated cultures per 10^6 lymphocytes were 1,867 ± 183 preinoculation, 1,481 ± 286 48 h postinoculation, and 1,631 ± 211 2 weeks postinoculation. No significant differences were noted among these values. Similarly, lymphocyte cultures obtained from volunteers with Norwalk-induced disease had a somewhat increased response to 0.5 μg of PHA per ml in the 48-h postinoculation sample when compared with preinoculation levels ($P < 0.05$) (Table 2). In lymphocyte cultures from eight volunteers who received the above inocula but who remained asymptomatic, no significant changes were observed in response to mitogens at the same three time points that were studied.

Addition of virus to lymphocyte cultures. Incubation of the infectious Norwalk or Hawaii filtrates, at dilutions ranging from 10^0 to 10^{-6}, with lymphocyte cultures did not elicit blastogenesis, nor did it enhance or inhibit blastogenic responses to PHA.

Lymphocytotoxic antibodies. Lymphocytotoxic antibodies were not detected in the sera of volunteers during acute illness or convalescence. In addition, incubation of lymphocytes from uninfected volunteers with sera from infected lymphopenic volunteers did not suppress or enhance blastogenic responses in the lymphocyte cultures.

Cortisol levels. Plasma samples (8 a.m.) obtained 48 h after inoculation contained mean cortisol levels which were within normal limits in ill (13.43 ± 1.04 μg/ml) and well (12.82 ± 1.10 μg/ml) volunteers.

DISCUSSION

In previous studies in man, transient lymphopenia has been described in infections with poliovirus (36), coxsackie A (10), rhinovirus (10), yellow fever virus (41), varicella (19), rubeola (4), rubella (18), Colorado tick fever (21), and influenza (17). Studies of circulating lymphocyte subpopulations during viral infections have been limited to infections outside of the gastrointestinal tract. Those studies have resulted in varied and occasionally conflicting findings in numbers of T and B cells (2, 29, 34, 43; B. S. Criswell and R. B. Couch, Fed. Proc. 34:948, 1975; M. A. Scheinberg, N. R. Blacklow, T. A. Parrino, F. B. Rose, and E. S. Cathcart, Clin. Res. 23:311A, 1975; J. D. Thorley, J. W.

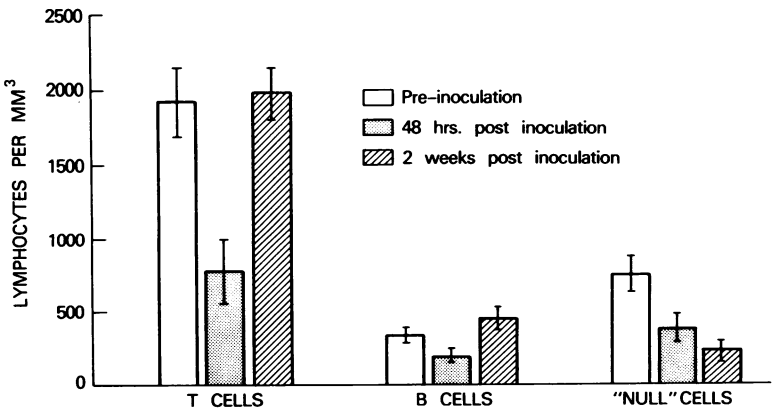

FIG. 3. *Circulating lymphocyte subpopulations in Hawaii agent-induced gastroenteritis. Height of bars represents mean ± SEM cell counts.*

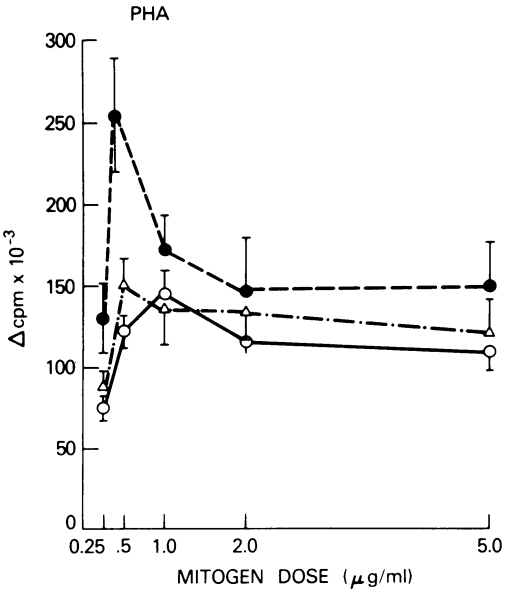

FIG. 4. *Blastogenic responses of phytohemagglutinin stimulated peripheral lymphocytes from volunteers with Hawaii agent-induced gastroenteritis. Values represent mean ± SEM counts per minute in stimulated lymphocytes minus the counts per minute in unstimulated lymphocytes in 10^6 cells, at varying concentrations of PHA. Symbols: ○, Preincubation; ●, 48 h postinoculation; △, 2 weeks postinoculation.*

Smith, and J. P. Sanford, Clin. Res. 22:455A, 1974), as well as in responses of lymphocytes to blastogenic stimuli (12, 24, 30–33, 35, 45). Interpretation of several of the above studies is made difficult by the absence of documentation of a specific viral infection (29, 34, 43; Thorley et al., Clin. Res. 22:455A, 1974). The disparity of the findings in other investigations may be partly attributed to the different viral agents which were studied or to the varying time intervals after infection at which samples were obtained (2; Criswell et al., Fed. Proc. 34:948, 1975; Scheinberg et al., Clin Res. 23:311A, 1975).

In the current investigations, the ability to study experimentally induced disease in normal volunteers enabled us to examine the effects of a specific viral illness upon circulating lymphocytes at specified time intervals after infection, under closely controlled conditions. In this setting, acute viral gastroenteritis induced by the Norwalk and Hawaii agents resulted in a marked, though transient, lymphopenia which occurred 48 h after inoculation and which was temporally associated with acute illness. The lymphopenia affected all lymphocyte subpopulations (T, B, and null cells). The mean percentage decrease in T cells appeared to be somewhat greater than the decrease in B cells, but the values were not statistically significantly different in the small number of volunteers studied. The lymphocytes that remained in the circulation responded normally or even supranormally to mitogenic stimulation with PHA and Con A. The preservation of blastogenic responses is consistent with the fact that there were no significant differences in the percentage of circulating T cells at the time points studied, despite an absolute lymphopenia 48 h postinoculation. Since all lymphocyte cultures were reconstituted to contain equivalent numbers of lymphocytes, the total number of T cells remained generally constant in the assays of blastogenesis.

The mechanisms that are responsible for the lymphopenia observed in these studies are unknown. In a previous study, antibody-associated lymphocytotoxin was demonstrated in 20 of 81 patients with lymphopenia after viral infection (20). In the current studies, lymphosuppressive factors, including antilymphocyte antibodies, could not be detected in the sera of lymphopenic volunteers. Several lines of evidence suggest that the above-described lymphopenia in acute viral gastroenteritis may be related to the viral infection itself, rather than being merely a nonspecific (stress) response to the acute illness. First, the magnitude of the lymphopenia was not correlated with either the severity or type (i.e., vomiting or diarrhea) of clinical illness. Second, the 8 a.m. plasma cortisol levels during acute illness were within normal limits. Third, two additional volunteers not described here, one with Norwalk- and the other with Hawaii-induced disease, experienced only slight anorexia or abdominal cramps without vomiting or diarrhea. Because of the presence of symptoms but the absence of

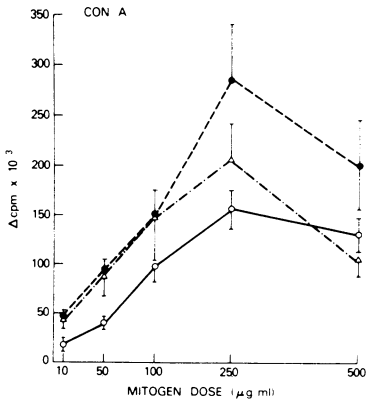

FIG. 5. *Blastogenic responses of Con A-stimulated peripheral lymphocytes from volunteers with Hawaii agent-induced gastroenteritis (details as in Fig. 4). Symbols: ○, Preinoculation; ●, 48 h postinoculation; △, 2 weeks postinoculation.*

TABLE 2. *Blastogenic response of phytohemagglutinin (PHA)-stimulated peripheral lymphocytes from volunteers with Norwalk agent-induced gastroenteritis*

Time of sample	No. of volunteers	Δcpm[a]	
		PHA[b] (0.5 μg/ml)	PHA[b] (1.0 μg/ml)
Preinoculation	9	98,667 (±8,876)	116,243 (±11,243)[c]
48 h postinoculation	9	162,631 (±23,892)[d]	148,861 (±18,432)[e]
2 weeks postinoculation	9	111,987 (±14,248)	105,281 (±16,286)[f]

[a] Counts per minute of incorporated [H³]thymidine in PHA-stimulated cultures minus counts per minute in unstimulated cultures in 10^6 cells. Data are given as mean (± standard error of the mean).

[b] Concentration of PHA in cultures.

[c] Counts per minute in unstimulated cultures: 1,723 ± 236.

[d] Significantly different from preinoculation value at PHA concentration of 0.5 μg/ml ($P < 0.05$).

[e] Counts per minute in unstimulated cultures: 1,646 ± 211.

[f] Counts per minute in unstimulated cultures: 1,411 ± 291.

objective signs of illness, they could not be classified as definitely ill or well and were, therefore, excluded from the above analyses. Both volunteers demonstrated antibody rises to the homologous agent as measured by immune electron microscopy (9, 26) and presumably underwent subclinical infection. Both volunteers also manifested the typical lymphopenia described above. Although this evidence suggests that the lymphocyte alterations seen in viral gastroenteritis may be a result of interaction with the virus itself or with a product of the viral infection, no in vitro interaction could be demonstrated between cultured lymphocytes and the relatively unpurified preparations of Norwalk and Hawaii agents that are currently available.

The time course of the transient lymphopenia, with its rapid onset and short duration, and the absence of circulating antilymphocyte factors are consistent with the development of an acute redistribution of circulating lymphocytes to extravascular sites, followed by a rapid replenishment of intravascular lymphocytes. Redistributions of circulating lymphocytes have been described in chronic lymphocytic leukemia (13), after corticosteroid administration in rodents (11, 28) and in tumor-bearing mice (14). In addition, Newcastle disease virus infection in mice and rats has resulted in a lymphopenia which has been attributed to redistribution of lymphocytes with a postulated viral-induced membrane alteration (44). The suggested redistribution of circulating lymphocytes in viral gastroenteritis coincides with the onset and resolution of clinical illness and parallels the development of intestinal histopathology of infection with Norwalk and Hawaii agents which has been described previously (1, 9, 37, 38). This histopathology appears to involve primarily the jejunal mucosa and consists of blunted villi with a mononuclear (and some polymorphonuclear leukocyte) infiltration in the lamina propria. This infiltration has been noted within 48 h after administration of the infectious inocula and resolves during convalescence (1, 9). The histopathology has also been reported occasionally with subclinical illness (37, 38). Thus, the depletion of circulating lymphocytes temporally coincides with the accumulation of lymphocytes in the jejunal mucosa. These observations suggest that the lymphopenia noted in the current studies may occur as a result of the redistribution of circulating lymphocytes to extravascular sites and, in particular, to the site of viral infection in the gut.

Although the above data were derived solely from examination of acute viral infections of the gastrointestinal tract, it is possible that similar mechanisms may be responsible for the transient lymphopenia reported during common, acute viral infections of other sites (10).

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