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Cytotoxic and Immunoregulatory Function of Intestinal Lymphocytes in *Chlamydia trachomatis* Proctitis of Nonhuman Primates

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To study the role of natural killer cells and immunoregulatory T cells in the pathogenesis of proctitis due to *Chlamydia trachomatis* (L2 serovar), lymphocytes were obtained from the rectal mucosa and other sites of nonhuman primates and studied by using phenotypic and functional assays. In animals with lymphogranuloma venereum (LGV) proctitis, the percentage of lymphocytes with the natural killer cell phenotype (Leu-11$^+$) was not significantly higher at any site in LGV infection, and natural killer cell function of lymphocytes isolated from the rectum was lower during LGV infection. This was not due to the suppressive effect of factors in serum, rectal lymphocytes, or LGV elementary bodies. In studies of regulatory T cells, the Leu-3$^+$/Leu-2$^+$ ratio was lower in the peripheral blood and the spleen during LGV infection, but the ratio did not decrease in lamina propria T cells. Both peripheral blood and rectal lymphocytes had higher helper T-cell function for polyclonal immunoglobulin G (IgG) synthesis in pokeweed mitogen-stimulated cultures 2 weeks following LGV infection. Increased suppressor T-cell function for pokeweed mitogen-stimulated IgG synthesis was found only in the peripheral blood of animals 2 weeks after infection, but not in isolated rectal lymphocytes. These results indicate that in LGV proctitis natural killer cells are not an important component of the inflammatory infiltrate at the site of infection, and helper T-cell function increases in peripheral blood and rectal lymphocytes.

Chlamydia trachomatis is a common human pathogen that causes a variety of different diseases, including eye, genitourinary, and gastrointestinal infections. The biovars L1, L2, and L3 cause lymphogranuloma venereum (LGV), a form of granulomatous proctitis (35, 40); while this disease is often mild, it sometimes is associated with significant local or systemic invasion and may have important late sequellae, such as rectal fibrosis. As in other infectious diseases, the degree of tissue injury and complications are probably in part a result of the immune response against the inciting agent rather than cellular damage caused directly by the agent. Most information regarding the immune response to *C. trachomatis* has been derived from studies of eye or genitourinary infections in humans (5, 15, 27, 37) or eye, genitourinary, or pulmonary infections in animal models (4, 24, 29, 38, 47–51). In human LGV, the details of the cellular and humoral events that occur are unknown, particularly at the site of infection in the rectal mucosa. Quinn et al. (36) recently described a model of LGV proctitis in monkeys that closely resembles human LGV proctitis. Because the immune system of primates closely resembles that of humans (7, 9, 17, 31, 33), the immune mechanisms in primate LGV proctitis would be expected to closely resemble LGV proctitis in humans. Therefore, we used this model to examine the role of cellular immune events in LGV proctitis, with particular emphasis on changes in lymphocytes at the site of infection in the rectal mucosa. In this study two aspects of the immune response were investigated: the roles of natural killer cells (44) and immunoregulatory T cells.

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MATERIALS AND METHODS

Animals. Fresh heparinized venous blood and tissues were obtained from normal adult male *Macaca fascicularis* monkeys (Cynomolgus monkey). Infected animals were housed individually in laminar flow cages as described previously (36). Control animals were housed similarly but received no inoculation.

Media and reagents. RPMI 1640 medium with L-glutamine, heat-inactivated fetal calf serum, gentamicin, and HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) buffer were obtained from MA Bioproducts, Walkersville, Md. Penicillin, streptomycin, amphotericin B, and pokeweed mitogen (PWM) were obtained from GIBCO Laboratories, Grand Island, N.Y. Hanks balanced salt solution without Ca^{2+} or Mg^{2+} and phosphate-buffered saline were obtained from Biofluids Inc., Rockville, Md. Ficoll-Hypaque solution was obtained from Litton Bionetics, Kensington, Md. Percoll was obtained from Pharmacia, Uppsala, Sweden. EDTA, *dl*-dithiothreitol, DNase, soybean trypsin inhibitor, and 2-mercaptoethanol were obtained from Sigma Chemical Co., St. Louis, Mo. Collagenase (*Clostridium histolyticum* type III), purified monkey immunoglobulin G (IgG), goat anti-monkey IgG, and fluorescein-conjugated goat anti-mouse IgG were obtained from Worthington Diagnostics, Freehold, N.J. Monoclonal antibodies included anti-human sheep erythrocyte receptor antibody (CD2, 9.6 clone), which was obtained from New England Nuclear Corp., Boston, Mass., and anti-Leu-2 (CD8), anti-Leu-3 (CD4), and anti-Leu-11 (CD16), which were obtained from Becton Dickinson and Co., Mountain View, Calif. Sodium [^{51}Cr]chromate (1 mCi/ml) was obtained from New England

Nuclear. Elementary bodies of *C. trachomatis* serovar L2 were prepared from lysates of infected McCoy cell lines and adjusted to a final concentration of 10^6 infectious units per ml.

Animal inoculation. Animals were infected by intrarectal submucosal inoculation under direct vision with 10^6 infectious units of LGV serovar L2. Active infection was confirmed by the presence of positive cultures from rectal swabs and detectable anti-LGV antibody in serum (determined by enzyme-linked immunosorbent assay) in all animals. Tissue and blood samples were obtained from animals in three groups of five animals each; this included samples from control animals and from infected animals obtained 2 and 6 weeks after inoculation. One animal each was inoculated at weekly intervals for 3 or 5 weeks and was sacrificed 1 week after the last inoculation.

Lymphocyte preparations. Peripheral blood lymphocytes (PBLs) were obtained by centrifugation of heparinized venous blood on Ficoll-Hypaque gradients. Spleen and mesenteric node lymphocytes were obtained by teasing spleens through wire mesh and centrifugation on Ficoll-Hypaque. Lymphocytes were isolated from the intestinal lamina propria of the ileum or the colon as described previously (22). Approximately 10 cm of right colon or rectum was removed, washed in phosphate-buffered saline, and cut into fragments (0.5 cm^2) in Ca^{2+}- Mg^{2+}-free Hanks balanced salts solution containing 10% fetal calf serum, 100 U of penicillin per ml, 1 mM EDTA, 25 mM HEPES buffer, 5×10^{-5} M 2-mercaptoethanol, and 1 mM *dl*-dithiothreitol. After a 15-min incubation at room temperature, the fragments were incubated in the same medium without dithiothreitol in plastic Erlenmeyer flasks on an orbital shaker at 37°C for 1 h, after which the supernatant, which contained primarily epithelial cells, was discarded. This procedure was repeated until the supernatant was clear. Histologic sections of these fragments showed that they were completely free of the epithelial cell layer, including intraepithelial lymphocytes. The fragments were then incubated in RPMI 1640 medium containing 10% fetal calf serum, HEPES buffer, antibiotics, 2-mercaptoethanol, 0.01% collagenase, 0.01% DNase, and 0.01% soybean trypsin inhibitor overnight at 37°C on an orbital shaker. The cells were then washed once in 25% isotonic Percoll solution; this procedure removed most debris and dead epithelial cells. The resulting cell preparation was centrifuged on Ficoll-Hypaque. The resultant lymphocyte preparations contained greater than 90% viable lymphocytes.

Immunofluorescence studies. Many anti-human lymphocyte monoclonal antibodies cross-react with surface glycoproteins on monkey lymphocytes (7, 9, 17, 31, 33). Furthermore, the monkey lymphocytes that react with particular monoclonal antibodies have the same function as their human counterparts (22, 31). Therefore, attempts were made to characterize the lymphocytic infiltrate in LGV proctitis by staining tissue sections with monoclonal antibodies by an immunoperoxidase method (Vectastain; Vector Laboratories, Burlingame, Calif.). However, considerable false-positive staining was found to be present by this method; this was due to the presence of a high endogenous peroxidase content of the normal gastrointestinal mucosa in monkeys. Thus, an alternative method was used to determine the types of lymphocytes that were present in the rectal mucosa in LGV proctitis. Lymphocytes were isolated from the intestinal mucosa by previously developed methods (22), and the phenotypes of the isolated lymphocytes were then determined by using a fluorescence-activated cell sorter. This method does not alter lymphocyte phenotype or function and has the advantage of greater sensitivity and objectivity compared with in situ histochemical methods.

Lymphocyte preparations were treated with saturating concentrations of the following monoclonal antibodies for 30 min at 4°C: anti-Leu-2 (fluorescein conjugate), anti-Leu-3 (fluorescein conjugate), anti-Leu-11 (fluorescein conjugate), and 9.6 (anti-human sheep erythrocyte receptor). Lymphocytes treated with 9.6 were then incubated for an additional 30 min with fluoresceinated goat anti-mouse IgG. Controls included unstained lymphocytes and lymphocytes treated with fluoresceinated goat anti-mouse IgG alone. A total of 10^4 cells were analyzed by using a fluorescence-activated cell sorter equipped with an argon laser (FACS II; Becton Dickinson). Fluorescence data were analyzed with a Digital Electric Corp. PDP-11/34 computer by using programs provided by Thomas Chused, National Institute of Allergy and Infectious Diseases, Bethesda, Md.

Cytotoxicity assays. Assays for natural killer cell function were carried out as described previously (22). In brief, 2×10^6 K562 target cells were labeled by incubation with 200 μCi of sodium [^{51}Cr]chromate for 30 min, after which the target cells were washed extensively in medium. Cytotoxicity assays were carried out by incubating lymphocytes with 5×10^3 target cells at various ratios in 96-well U-bottom microtiter plates for 18 h at 37°C. The culture medium consisted of RPMI 1640 with 10% fetal calf serum, antibiotics, and HEPES buffer, as described above. At the end of the assay culture supernatants were harvested with harvesting frames (Skatron Inc., Lier, Norway) and counted in a gamma scintillation counter. Background release from target cells was always less than 20%. Maximal release of ^{51}Cr was determined by treatment of target cells with detergent. Assays were carried out in quadruplicate.

Immunoglobulin synthesis in vitro. PWM-stimulated IgG synthesis in vitro was determined as described previously (20, 23). In brief, lymphocytes from blood or tissues were separated into T-cell- and B-cell-enriched fractions by rosetting with sheep erythrocytes. T-cell fractions contained greater than 95% sheep erythrocytes–lymphocytes as determined by flow cytometry with 9.6 monoclonal antibody. A total of 10^5 B-cell-enriched cells from spleens were cultured with an equal number of various combinations of autologous T cells from different sites in 96-well round-bottom microtiter plates with a final volume of 0.22 ml in RPMI 1640 medium with 10% fetal calf serum, HEPES buffer, antibiotics, and an optimal dilution of PWM (1:100). Cultures were carried out in duplicate. After 10 days of culture, culture supernatants were harvested and stored at −20°C prior to assay. Supernatant monkey IgG was determined in duplicate by radioimmunoassay as described previously for human immunoglobulins (20, 23).

Calculations. Values are reported as either the median or mean ± standard error, as indicated. The significance of differences was determined by using the Kruskal-Wallis test to compare three or more groups of data, and the Mann-Whitney U test was used for the comparison of two groups. Cytotoxicity values were expressed as follows: specific lysis = [100 × (experimental counts − background counts)/(maximal counts − background counts)]. Suppression of immunoglobulin synthesis was determined as follows: % suppression = [100 × (IgG in control culture − IgG in test culture)/(IgG in control culture)].

RESULTS

In this study mild proctitis due to LGV serovar L2 was produced in animals by a single intrarectal inoculation. By 2

TABLE 1. Percentage of Leu-11+ lymphocytes and natural killer cell activity in LGV infection

Lymphocyte source	% Leu-11+ cells[a]:			P^c	Natural killer cell activity[b]:			P^c
	In controls	At 2 wk	At 6 wk		In controls	At 2 wk	At 6 wk	
Peripheral blood	10.1	9.0	3.9	NS	70.5	58.3	31.1	NS
Spleen	5.9	6.1	3.2	NS	36.5	58.5	36.8	NS
Colon	12.5	8.0	6.1	NS	23.0	11.7	12.8	<0.05
Rectum	8.5	16.5	5.5	NS	20.6	12.9	5.5	<0.05

[a] Median values for the percentage of lymphocytes stained with Leu-11, as determined by flow cytometry.
[b] Natural killer activity against K562 target cells, expressed as median values of percent specific lysis at a lymphocyte/target ratio of 50:1. There were five animals in each of three groups (controls and animals from which samples were taken at 2 and 6 weeks after infection).
[c] $P < 0.05$, as determined by the Kruskal-Wallis test. NS, Not significant.

weeks slight granularity, erythema, and friability of the mucosa was present in the rectum, and lymphoid nodules were often visible. By 6 weeks no gross abnormalities were visible on proctoscopic examination, although rectal cultures were still positive for LGV. Histological features of proctitis consisted of mild inflammation characterized by focal accumulation of lymphocytes and plasma cells, microscopic focal ulceration, and crypt abscesses. Granulomas were not found. The lesions observed were present throughout the rectum and did not involve only the areas receiving direct inoculation. The morphology for the control tissues obtained from the proximal right colon was normal. These findings are in agreement with those published previously (36).

Effect of LGV infection on natural killer cell function. In previous studies of lamina propria lymphocytes, very little natural killer cell activity could be demonstrated with either human lymphocytes or monkey lymphocytes by the 4-h Cr release assays against targets such as K562. However, if the Cr release assays were carried out for 18 h, significant natural killer cell activity could be demonstrated in the intestinal lymphocyte preparations, although the cytotoxic activity of intestinal lymphocytes was still significantly lower than that of PBLs or spleen lymphocytes (22). Furthermore, the proportion of lymphocytes that was reactive with anti-Leu-11 is low in lamina propria in both humans (12) and monkeys (22). In this study the proportion of rectal lymphocytes that was reactive with anti-Leu-11 was not significantly different when infected animals were compared with uninfected controls (Table 1). The proportion of Leu-11+ rectal lymphocytes in an animal inoculated five times was 2.5%. When the capacity of isolated lymphocytes to kill K562 target cells was tested in LGV-infected monkeys, it was found that the natural killer activity of rectal lymphocytes was lower 2 weeks after infection (Table 1).

This result was surprising because intracellular infections often stimulate natural killer activity (2, 16, 18, 41, 42, 46). Therefore, the possibility that the natural killer cell activity of intestinal lymphocytes was inhibited during the infection was investigated. Because lymphocytes in the intestinal lymphocyte preparations might suppress natural killer cell function, lymphocytes from the rectums of normal or infected animals were added to autologous PBLs with significant natural killer function (Table 2). However, no evidence of suppression by intestinal cells was found. Similarly, to determine if serum from infected animals inhibited natural killer function, serum was added to autologous PBLs, but again there was no evidence of inhibition (data not shown). Finally, when LGV elementary bodies were added to natural killer cell assays, there was no evidence of direct inhibition of natural killer cell function by the elementary body preparation used to inoculate the animals (Table 3).

Effect of LGV infection on T-cell immunoregulatory function. Because immunoregulatory T cells may have a role in modulating the inflammatory response in LGV proctitis, the immunoregulatory function of T lymphocytes that were isolated from different sites was studied. The proportion of T cells, which was determined by staining lymphocytes with an anti-human sheep erythrocyte receptor monoclonal antibody, was not significantly different when lymphocytes from the rectum of infected animals were compared with those from uninfected controls. However, there was an increase in the proportion of T cells in the spleens of infected animals (Table 4). In studies of T-cell subpopulations, it was found that there was a significant increase in the proportion of Leu-2+ (suppressor/cytotoxic) T cells in spleens; the proportion of Leu-2+ PBLs was higher in LGV-infected animals, but the values were not significantly higher than those in controls. The proportion of Leu-2+ lymphocytes did not change significantly in colon or rectal lymphocytes. The proportion of Leu-3+ (helper/inducer) T cells was significantly higher only in colon lymphocytes 2 weeks after

TABLE 2. Effect of rectal lymphocytes on natural killer cell activity of autologous PBLs

Rectal/PBL ratio[a]	% Specific lysis[b]:		
	In controls	At 2 wk	At 6 wk
4:1	54.5 ± 14.5	44.8 ± 4.5	46.3 ± 6.4
2:1	52.5 ± 11.3	40.8 ± 4.5	46.1 ± 9.7
1:1	54.4 ± 10.4	32.3 ± 13.9	44.6 ± 7.5
0.5:1	55.0 ± 11.0	44.2 ± 7.1	42.6 ± 6.9
0:1	53.4 ± 4.3	45.1 ± 9.9	44.4 ± 9.1

[a] Autologous rectal lymphocytes were added to PBLs at the indicated ratios.
[b] Mean values for percent specific lysis using K562 target cells and a fixed peripheral blood/K562 target cell ratio of 50:1. There were five animals in each group (controls and animals from which samples were taken at 2 and 6 weeks after infection).

TABLE 3. Effect of LGV elementary bodies on natural killer cell activity

Lymphocyte source and LGV	% Specific lysis at the following effector/target ratios[a]:	
	50:1	25:1
Peripheral blood		
−	47.4 ± 5.0	32.8 ± 5.5
+	44.8 ± 10.4	24.8 ± 6.6
Rectum		
−	14.8 ± 8.1	12.3 ± 7.5
+	11.5 ± 3.1	9.5 ± 3.2

[a] Mean values for percent specific lysis of K562 target cells at the indicated effector/target ratios in the absence or presence of 10^5 LGV elementary bodies per ml. Values are for groups of four animals each.

TABLE 4. T-cell subpopulations in LGV infection[a]

Lymphocyte source and monoclonal antibody	% Lymphocytes[a]:					P^b
	In controls	At 2 wk	At 6 wk	When multiply infected:		
				Three times	Five times	
Peripheral blood						
Pan-T	69.0	70.1	79.8		80.7	NS
Leu-2	35.0	39.2	50.0	45.1	69.2	NS
Leu-3	30.0	9.1	32.3	36.4	25.0	NS
Leu-3/Leu-2	0.91	0.32	0.66	0.81	0.36	<0.05
Spleen						
Pan-T	51.0	70.0	80.0		75.7	NS
Leu-2	22.0	41.7	56.2	46.2	59.1	NS
Leu-3	14.0	9.6	23.5	24.5	24.1	<0.05
Leu-3/Leu-2	0.82	0.31	0.42	0.53	0.41	<0.05
Colon						
Pan-T	77.5	53.6	74.0			NS
Leu-2	37.9	36.0	45.7	33.9		NS
Leu-3	30.0	56.6	33.3	42.6		<0.05
Leu-3/Leu-2	0.80	1.31	0.73	1.26		<0.05
Rectum						
Pan-T	59.0	72.8	47.8		84.8	NS
Leu-2	39.2	47.8	57.3	37.2	35.6	NS
Leu-330.9	30.9	43.6	41.2	19.2	57.1	NS
Leu-3/Leu-2	0.77	0.88	0.77	0.52	1.60	NS

[a] Median values for percentage of lymphocytes stained with the indicated monoclonal antibodies, as determined by flow cytometry. There were five animals in each of three groups (controls and animals from which samples were taken at 2 and 6 weeks after infection), and one animal each was inoculated at weekly intervals three or five times.

[b] The significance of differences in the controls and animals from which samples were taken at 2 and 6 weeks was tested by the Kruskal-Wallis test. NS, Not significant.

infection. These changes were reflected in changes in the Leu-3/Leu-2 ratios. In PBLs and spleen lymphocytes the Leu-3/Leu-2 ratio was significantly lower 2 weeks after infection, but in rectal lymphocytes the ratio was higher in infected animals. Similar changes were found in the multiply infected animals.

The capacity of T cells to provide help was tested by coculturing T cells with B cells from a single source, the spleen, and PWM. Spleen B cells in the absence of added T cells had low levels of IgG synthesis (Table 5), although it is interesting that 2 weeks after infection IgG synthesis by unstimulated spleen B cells was higher in the animals, suggesting the presence of preactivated B cells in the spleens of these animals. Peripheral blood T cells from all three groups provided significant help with IgG synthesis. Despite the decline in the Leu-3/Leu-2 ratio after 2 weeks of infection, T-cell help was higher in both groups of infected animals compared with that in controls. T cells from the intestinal mucosa of all groups of animals provided less help than peripheral blood T cells. There was no significant change in helper function of T cells isolated from the uninvolved part of the colon. However, T cells isolated from the rectums of animals 2 weeks after infection had higher levels of help compared with those from controls.

Changes in the antigen-nonspecific suppressor function of T cells was also examined during LGV infection. To study this, T cells from different sites were added to test cultures that were capable of producing IgG, namely, cultures containing autologous spleen T cells, B cells, and PWM. Suppression of IgG synthesis by autologous T cells was found only in peripheral blood T cells of animals 2 weeks after LGV infection (Table 6). T cells from the colon or rectum did not suppress IgG synthesis, but they actually enhanced IgG synthesis further. These functional results corresponded to changes in the Leu-3/Leu-2 ratio noted above, in which it was found that the ratio was lower in PBLs but was higher in the intestinal mucosal lymphocytes during LGV infection.

DISCUSSION

One of the objectives of this study was to determine the possible role of antigen-nonspecific cytotoxic cells, in particular, natural killer cells, in the effector phase of the inflammatory response in the intestinal mucosa during LGV infection. Because LGV is an intracellular infection, it seemed likely that natural killer cells might be activated by this particular infection, as has been found in other types of intracellular infections (2, 16, 18, 41, 42, 46). Furthermore, there are a number of ways in which cytolytic cells might play a role in LGV, including direct lysis of infected epithelial cells (either de novo or following lymphokine activation [12, 22]) or by modulation of the function of other immune cells (3, 43). A second objective was to determine the role of immunoregulatory T cells in modulating the inflammatory response in the intestinal mucosa. The results of these studies might provide insight into human intestinal diseases that are characterized by chronic inflammation, particularly those with pathological and clinical similarities to LGV, such as Crohn's disease (10).

In previous studies of normal nonhuman primates, we found that although natural killer cells are present in the intestinal mucosa, their functional activity is significantly less than that in peripheral blood (22). These findings are in agreement with results of previous studies of human intestinal lymphocytes, in which little natural killer cell activity has been found (13, 28). In this study, when the natural killer cell activity of isolated lymphocytes was determined by measuring their capacity to kill K562 target cells in animals with LGV infection, it was shown that natural killer cell activity is lower in intestinal lymphocytes after 2 weeks of infection. Because lymphokine-activated killer cells are able to kill some of the same target cells used in classical natural killer cell assays, in particular K562 cells, the observation in this study that there is little lytic activity of freshly isolated intestinal lymphocytes in LGV proctitis indicates that lymphokine-activated killer cell activity is also low in the intestine during this infection. Furthermore, there were no significant changes in the proportion of cells with the Leu-

TABLE 5. Helper T-Cell function in LGV infection

Source of T cells added to spleen B cells and PWM[a]	IgG (ng/ml)[b]:			P^c
	In controls	At 2 wk	At 6 wk	
None added	110	286	45	NS
Peripheral blood	1,200	2,699	2,355	<0.05
Colon	410	520	189	NS
Rectum	547	1,588	619	<0.05

[a] T cells were isolated from peripheral blood, colon, or rectum of groups of five animals each in three groups (controls and animals from which samples were taken at 2 and 6 weeks after infection). Spleen B cells (10^5) were cultured either alone or with autologous T cells (10^5) and PWM for 10 days, after which supernatant IgG was determined.

[b] Median values for each group.

[c] The significance of differences between groups was determined by the Kruskal-Wallis test. NS, Not significant.

TABLE 6. Suppressor T cell function in LGV infection

Source of cells added to spleen B cells, spleen T cells, and PWM[a]	% Suppression[b]:			P[c]
	In controls	At 2 wk	At 6 wk	
Peripheral blood	−58	42	−41	<0.05
Colon	−0.5	−129	−35	NS
Rectum	−90	−127	−135	NS

[a] T cells from peripheral blood, colon, or rectum were isolated from five animals in each of three groups (controls and animals from which samples were taken at 2 and 6 weeks after infection). T cells (10^5) were cultured with equal numbers of autologous spleen T cells, spleen B cells, and PWM for 10 days, after which supernatant IgG was determined, and suppression was calculated by comparing cultures containing spleen T cells and B cells alone (see text). Negative values for suppression indicate enhancement rather than suppression.

[b] Median values for each group.

[c] The significance of differences between groups was determined by the Kruskal-Wallis test. NS, Not significant.

11^+ phenotype, a phenotype that is thought to include most cells with natural killer cell activity. Finally, there was no evidence that factors in serum, intestinal lymphocytes, or LGV elementary bodies directly inhibited natural killer cell activity, as has been found to occur during infection with some intracellular organisms (26). Thus, it appears that natural killer cells do not play an important effector role in the inflammatory response in LGV infection. This conclusion is consistent with results of studies done with *nu/nu* mice, which have increased susceptibility to infection with murine *C. trachomatis* (48). Because *nu/nu* mice have functional natural killer cells, this finding also indicates that natural killer cells alone are not sufficient to confer resistance to infection.

To characterize the function of immunoregulatory T cells in LGV proctitis, studies of both T-cell phenotype and function were carried out. With regard to the phenotypic characterization of the Leu-2^+ (CD8, suppressor/cytotoxic) and Leu-3^+ (CD4, helper/inducer) lymphocytes, we found that the percentage of Leu-2^+ cells was higher in PBLs and spleen lymphocytes, but not in intestinal lymphocytes. Furthermore, the percentage of Leu-3^+ cells was slightly higher in colon and rectal lymphocytes in the LGV-infected animals than in control animals. These findings were reflected in the changes in the ratio of Leu-3- to Leu-2-positive cells; the ratio fell in peripheral blood and spleen, and there was a slight rise in the lamina propria lymphocytes during LGV infection. Thus, it appears that during LGV infection the changes that occur in the peripheral blood do not reflect the changes that occur at the site of infection.

The phenotypic changes in Leu-2^+ and Leu-3^+ lymphocytes were correlated with the immunoregulatory function of T cells in PWM-stimulated cultures. The results of this study indicate that while both helper and suppressor function increased in the peripheral blood of animals 2 weeks after LGV infection, the lymphocytes that were isolated from the rectal mucosa demonstrated only helper function but no increase in suppressor T-cell function. Interestingly, although the Leu-3/Leu-2 ratio was lower in peripheral blood 2 weeks after infection, helper T-cell function was higher, suggesting that at this time there is preferential activation of helper T cells rather than suppressor T cells.

The finding of antigen-nonspecific suppressor cells in the peripheral blood during intestinal inflammation due to LGV proctitis is of interest for several reasons. This finding is similar to those obtained in a primate model of *C. trachomatis* eye infection, in which it was found that following systemic and ocular immunization, lymphocyte proliferative responses were inhibited by Leu-2^+ T cells in peripheral blood (51). There is substantial evidence that mucosal exposure to specific antigens via the gastrointestinal tract can result in activation of suppressor T cells that inhibit systemic immune responses to that antigen (1, 8, 32, 34, 39). Therefore, it is possible that the presence of circulating antigen-nonspecific suppressor T cells in LGV proctitis reflects activation of this normal immune mechanism of oral tolerance. On the other hand, a significant increase in the percentage of cells with the Leu-2^+ phenotype and an increase in antigen-nonspecific suppressor cell function were not found at the site of inflammation in the rectum during LGV infection. These findings are similar to those from studies of lymphocytes from patients with inflammatory bowel disease, in which there was evidence of increased antigen-nonspecific suppressor activity in peripheral blood (23) but no evidence of activated suppressor cells in the intestine (11, 20). Thus, results of this study indicate that an increase in the activity of circulating suppressor T cells may occur during intestinal inflammation, but these suppressor cells do not necessarily appear at the site of inflammation.

The question remains as to what immune mechanisms are important in the inflammatory response in *Chlamydia* infections. As mentioned above, *nu/nu* mice have increased susceptibility to infection with the murine pneumonitis agent (48). The immune deficiency of congenitally athymic mice is not total. Recently, it has been shown that these animals are profoundly deficient in the L3T4^+ T-cell subset (30), which is thought to be equivalent to the Leu-3^+ subset of T cells in humans and primates. This subpopulation of T cells is known to play a central role in immune responses because they provide a helper function for antibody responses and are necessary for activation of cytolytic T cells. Because it is still uncertain whether antibodies against *Chlamydia* antigens are protective (6, 14, 25, 29, 45), it may be that a more important function of Leu-3^+ T cells than providing help for antibody production is to activate antigen-specific cytolytic T cells. Cells with the Leu-3^+ monoclonal antibody phenotype are prominent in the intestinal lamina propria, and cells with the phenotype of cytolytic effector cells have been identified in the human (21) and primate intestinal lamina propria. In other models of intestinal infection, it has been shown that enteric exposure to viruses can result in virus-specific cytolytic T cells in gut-associated lymphoid cells (19). Thus, it is likely that Leu-3^+ T cells, which were found to be present in increased proportions and to have an increased helper function in the rectal mucosa during LGV proctitis, play a central role in the immune response in this infection both in antibody production and in activation of cytolytic T cells.

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