Treponemal Infection Specifically Enhances Node T-Cell Regulation of Macrophage Activity

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Hamsters experimentally inoculated in the inguinal region with *Treponema pallidum* subsp. *endemicum* develop considerable pathology at that site. We examined the cell populations from these inguinal lymph nodes to determine their intercellular responses to infection. In vitro, syphilitic-node T cells markedly suppressed C3b receptor-mediated ingestion (C3bMI) in syphilitic macrophages derived from sites both proximal and distal to the inoculation. This activity was more pronounced when node T cells rather than peritoneal T cells were used. When treponemal preparations or live treponemes were added to the coculture system, the suppression was specifically enhanced, whereas the addition of heterologous agents did not promote this effect. Syphilitic macrophages from either compartment cultured alone showed no significant inhibition of C3bMI. In parallel studies on syphilitic macrophages, we observed that the expression of Ia quickly became elevated and was sustained throughout the infection. Moreover, in vitro culturing of the syphilitic-node T cells with these macrophages did not alter this function. These observations suggest that the syphilitic node contains a subpopulation of T cells that can selectively suppress macrophage C3bMI activity and concurrently regulate their cellular response to treponemal infection.

The immunopathology of syphilitic infection involves several complex host-parasite interactions which may contribute to the chronicity of the disease (32, 50). Studies show that despite elevated immune responses, the infected host is usually unable to eliminate the organism completely (6, 44). Serum from some of these infected animals is treponemicidal in vitro, whereas in vivo it can passively confer protection to nonimmune recipients that are challenged with the autologous strain (2, 33). Many of these aspects are readily apparent in the hamster model system during *Treponema pallidum* infection. Some investigators suggest that the coating of treponemes with host protein macromolecules may allow the organism to evade phagocytosis and immune processing (1, 8). Other investigations imply that a defect in the macrophage (Mϕ) system may account for treponemal survival (12, 20). During early stages of the disease, investigators have observed lymph node hyperplasia and characterized the responsiveness of lymphocytes to infection (25, 41, 42). Subsequently, Bagasra and Damjanov have shown that although peritoneal Mϕ satisfactorily present treponemal antigens, node Mϕ are more effective in this function (4). Recently, studies with T-cell-enriched fractions from syphilitic animals demonstrated an inhibition of Mϕ C3b receptor-mediated ingestion (C3bMI) during infection (49). Both the propagation and enhancement of this activity would diminish the ability of the host to appropriately eliminate the treponeme. Collectively, these events prompted further investigation into the interactions occurring at the T-cell–Mϕ level. The current work focuses on the responses derived from T-cell–Mϕ interactions in the local area and at a site distal to infection. The results indicate that treponeme-induced node T cells specifically generate the strongest regulatory influence on the Mϕ response to infection. This activity selectively diminishes C3bMI, a major host defense mechanism, despite the concurrent enhancement of Mϕ Ia

expression. In vitro studies further show that the increase of Mϕ Ia surface markers is promoted by interactions between syphilitic-node T cells and Mϕ. Treponemal induction of a suppressive response would be advantageous to the parasite by enabling it to survive without killing the host. Therefore, selective modulation of node T-cell activity may elucidate the most prominent regulatory events and perhaps enhance host elimination of the treponeme.

MATERIALS AND METHODS

Animals. Inbred 10-week-old LSH/Ss Lak male hamsters were obtained from the Charles River Breeding Laboratories, Inc., Wilmington, Mass. Hamsters weighing 80 to 100 g were housed four per cage and given antibiotic-free food and water ad libitum.The animal quarters were regulated to maintain an ambient temperature of 18°C, which facilitates the development of cutaneous lesions (23). Each animal was shaved prior to treponemal infection and was maintained free of hair by being clipped twice a week.

Organisms and infection of hamsters. *T. pallidum* subsp. *endemicum*, formerly *T. pallidum* Bosnia A (40), was maintained by passage in LSH hamsters in our laboratory as previously described (49). At 3 to 4 weeks post intradermal infection, lymph nodes were aseptically removed and teased through 60-mesh stainless-steel screens into RPMI 1640 containing 10% fetal calf serum (C medium). The gross tissue debris was removed by allowing the preparation to settle for 5 min at unit gravity. Treponeme-containing suspensions were purified by the Percoll gradient density method as described by Hanff et al. (21). Treponemes were resuspended in C medium and enumerated by dark-field microscopy as described by Miller (28). Hamsters were intradermally infected at two sites in the shaved inguinal region by delivering 1×10^5 to 5×10^5 organisms suspended in 0.1 ml of C medium to each site. This treatment consistently produces chronic lesions that persist for 6 to 9 months (39).

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Cell harvest and Mϕ preparation. All hamsters were asphyxiated with CO_2 prior to aseptic harvest of peritoneal cells and lymph nodes. The peritoneal cavity of each sacrificed normal and treated hamster (infected or 3% thioglycolate stimulated) was surgically exposed, and 15 ml of C medium supplemented with 10 U of sodium heparin per ml was injected directly into the cavity. After a vigorous manual massage of the area, cell suspensions were aspirated with a 20-gauge needle into a 20-ml syringe. All cells were washed three times in phosphate-buffered saline (PBS)–10% FCS, counted, and resuspended in C medium containing 100 U of penicillin and 100 μg of streptomycin per ml. Cells from all animals were pooled within their appropriate groups. Surgically excised inguinal lymph nodes in cold PBS were carefully trimmed of excess tissue under a dissecting microscope. Nodes were teased over sterile 60-mesh stainless-steel screens, and preparations were collected over PBS. These suspensions were then aspirated and dispensed several times to disburse any clumps. They were subsequently incubated for 25 min with collagenase (Sigma Chemical Co., St. Louis, Mo.), washed, and treated for 15 min with DNase (Sigma). After three washes in PBS–10% FCS at 400 × g for 10 min (4°C), cells were counted and resuspended in C medium plus penicillin and streptomycin (as described above) supplemented with 5-hydroxybromouridine (Sigma) to inhibit fibroblasts. In experiments with live organisms, cells were cultured in C medium without antibiotics. Trypan blue exclusion studies routinely showed 90 to 94% viability in these preparations. Cell concentrations were adjusted to yield 10^5 Mϕ when plated on flame-sterilized 12-mm glass cover slips (Bellco glass, Inc., Vineland, N.J.) in 16-mm wells of 24-well sterile-plastic tissue culture plates (Costar, Cambridge, Mass.). Total and differential cell counts were performed on the cover slips with Wright-stained (Sigma) preparations and ocular grid analysis. After 1 h of incubation at 37°C with 5% CO_2 in a humidified environment, individual cover slips were removed with forceps, immersed in warm C medium, and washed three times by using gentle bursts of C medium. The nonadherent cells collected were enriched for T cells as described below and held on ice until used. The adherent cells were recounted to standardize monolayers and were identified as Mϕ by morphology, neutral-red uptake, and nonspecific esterase staining.

Isolation of hamster T cells. Enrichment of all nonadherent populations for T cells was performed as previously described by Tabor et al. (49). Pooled nonadherent-cell suspensions were overlaid on Ficoll-Hypaque gradients (density, 1.077; Pharmacia Fine Chemicals, Uppsala, Sweden) and centrifuged at 400 × g for 30 min at 25°C. Mononuclear cells free from debris were recovered at the interface, washed, counted for viability, and suspended in RPMI at 10^7 cells per ml. All these preparations were then fractionated by differential adherence to nylon wool columns. Effluent cells were washed, suspended in PBS–5% FCS, and decanted into goat anti-hamster immunoglobulin antiserum (Cooper Biomedical Inc., Malvern, Pa.)-coated panning flasks. Post incubation, the T-cell-enriched populations were collected and their character was verified by cytotoxic analysis with a 1/100 dilution of monoclonal anti-murine Thy 1.2 antibody (NEI-001; New England Nuclear Corp., Boston, Mass.) and a 1/10 dilution of guinea pig complement absorbed with fresh hamster erythrocytes and spleen cells. Control cells were incubated with antiserum, complement, or medium alone. T-cell supensions collected after the enrichment process contained 93% ± 3% T cells (mean ± standard error of the mean [SEM]).

Cocultivation of Mϕ with T lymphocytes. Cocultivation systems were prepared as previously described (49) with slight modifications. Briefly, fresh Mϕ explants from normal, 3% thioglycolate-treated, or 3-week-syphilitic hamsters were overlaid with T-cell suspensions. These preparations were incubated on a rocking platform (Bellco) at 3 cycles per min for 48 h at 37°C in 5% CO_2 in an humidified environment. In select experiments, *T. pallidum, Staphylococcus aureus,* or *Listeria monocytogenes* cells which had been passed in hamsters were washed three times with C medium, formalinized, and added directly to each well containing Mϕ and T cells. Additionally, one group received live washed treponemes. After incubation as described above, all Mϕ-containing cover slips were removed, washed with C medium three times to remove nonadherent cells, and assayed for activation. Experiments were designed so that the extent of activation could be assessed simultaneously for all groups. Mϕ cultured in medium alone served as control groups. There was no significant loss of Mϕ or death of T cells within the culture period. This fact determined by periodic removal of representative samples and evaluation of the cell numbers, esterase staining, and viability of both populations.

C3bMI assay. Mϕ activation was assessed by using the criterion of C3bMI activity as originally described by Bianco et al (13) with the slight modifications used by Tabor et al. (49). Briefly, washed sheep erythrocytes (E) were opsonized with a subagglutinating concentration of 19S immunoglobulin M (IgM) rabbit-anti E (Cordis Laboratories, Inc., Miami, Fla.) and fresh LSH hamster serum as a source of complement. Ingestion controls consisted of E opsonized with antibody alone and E alone to identify Fc or nonspecific ingestion, respectively. Triplicate slides were examined microscopically under oil immersion.

The percentage of Mϕ ingesting prepared erythrocytes multiplied by the average number of erythrocytes ingested per 100 Mϕ (ingestion index) was used to evaluate the extent of Mϕ activation. The results were reported as the EAC (sheep erythrocytes opsonized with antibody and complement) ingestion index minus the background, which did not exceed 4% of the EAC. Negligible ingestion of antibody-opsonized E or E alone was observed throughout the assays. This finding indicated that IgG antibody contamination or erythrocyte damage or both were minimal. The percentage of erythrocyte ingestions showed a direct correlation with the ingestion index throughout this study.

Determination of Ia^k antigen-bearing Mϕ. Hamster Mϕ readily express detectable major histocompatibility complex class II antigens (34, 35). The percentage of cells positive for this marker was determined by modifying the methods of Bagasra and Damjanov (4). Cover slips containing Mϕ were washed twice, and cells were overlaid with 50 μl of PBS–0.05% NaN_3 before being divided into two groups. One group was incubated with a 1/20 final dilution of fluorescein-conjugated mouse monoclonal antibody to Ia^k (broadly reactive alloantisera, A · TH Ia^k–anti-A · TL Ia^k; Accurate Chemical and Scientific Corp., Westbury, N.Y.). The other group received a fluorescein-conjugated mouse monoclonal IgG$_2$ antibody as a negative control. All assays were prepared in triplicate. After a 45-min incubation period at 4°C, the cells were washed three times, fixed, rinsed, and inverted over glycerol for evalution by fluorescence microscopy. At least 200 cells per cover slip from five random fields were examined. Background staining was always less than 3%.

Data analysis. Tests for statistical differences were per-

formed by using the Student t test. Values for the t statistic were calculated with data derived from experiments performed in triplicate, with a minimum of eight hamsters per experimental group.

RESULTS

Mφ derived from hamster lymph nodes or peritonea were induced in vivo to become activated either by using a phlogistic agent (i.e., thioglycolate) or by treponemal infection alone. Expression of this activity became apparent when the Mφ were assayed after their removal from the original in vivo host milieu (Table 1). Subsequently culturing these Mφ either alone or in the presence of naive T cells, regardless of tissue source, permitted Mφ to markedly enhance their utilization of the C3b receptor for ingestion. Conversely, Mφ derived from treponeme-infected hamsters demonstrated a significant reduction in C3bMI activity after coculture with T cells from syphilitic animals. Although this observation was readily discernible when Mφ and T cells were derived from either lymph node or peritoneum, a more pronounced effect was most apparent when the cell combination was derived exclusively from the node (Table 1). A similar effect on Mφ activation was also observed when thioglycolate-treated animals were studied under these conditions. However, the magnitude of the intertissue differences was considerably less than that demonstrated by the cells cocultured from the syphilitic animals alone (Table 1). Of tantamount interest in this study was the observation that when reputedly activated Mφ were cocultured with either node or peritoneal T cells from treponeme-infected animals, there was a significant decline in C3bMI activity regardless of the tissue source from which the Mφ was harvested (Table 1). Moreover, the intertissue differences previously observed between the node and peritoneal Mφ–T-cell systems from the syphilitic animals were completely ablated. The results also show that thioglycolate-treated Mφ cocultured
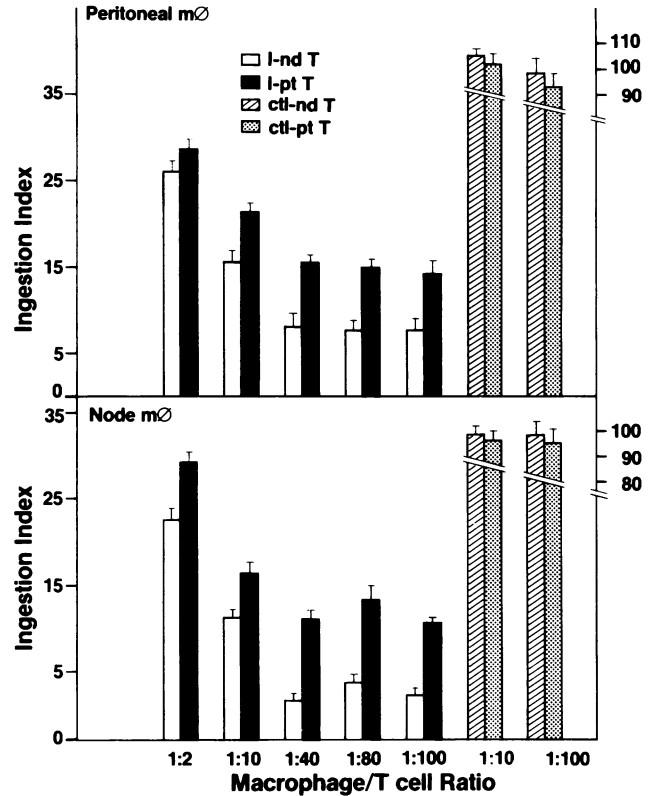

FIG. 1. Effect of varying Mφ-to-T-cell ratio on suppression of Mφ C3bMI. T cells from 3-week-syphilitic hamster lymph nodes (I-nd T) or peritonea (I-pt T) were cocultured with Mφ for 48 h before assay. T cells from normal nodes (ctl-nd T) or normal peritonea (ctl-pt T) were used as controls. Results represent the mean of experiments performed in triplicate ± SEM.

TABLE 1. Differential effect of lymph node and peritoneal T cells from syphilitic animals on Mφ C3bMI activity

T-cell tissue source[a] and Mφ host type[b]	Mφ phagocytic response[c]	
	Node	Peritoneum
Normal node		
Normal	7 ± 5	14 ± 4
Infected	58 ± 11	71 ± 5
Stimulated	98 ± 12	112 ± 9
Infected node		
Normal	14 ± 3	21 ± 3
Infected	20 ± 5	31 ± 7
Stimulated	46 ± 8	51 ± 3
Normal peritoneum		
Normal	12 ± 6	22 ± 8
Infected	63 ± 7	84 ± 9
Stimulated	113 ± 18	141 ± 12
Infected peritoneum		
Normal	11 ± 7	28 ± 4
Infected	42 ± 4	54 ± 5
Stimulated	64 ± 3	70 ± 8

[a] T cells were harvested from lymph nodes or peritonea of syphilitic or normal animals and were concultured in vitro with Mφ.

[b] Mφ were harvested from normal, syphilitic, or thioglycolate-stimulated animals.

[c] C3bMI index ± SEM. Experiments were performed in triplicate.

with T cells from syphilitic animals were similarly regulated (Table 1).

This investigation further demonstrated that all these activities were best manifested when an optimum Mφ-to-T-cell ratio was used. Although only a 1:2 (Mφ/T cell), ratio yielded a response, the most prominent effect on suppression appeared when a 1:40 ratio was used (Fig. 1). Subsequently increasing the T-cell quantity in this ratio did not appreciably augment the response. The best results were achieved by using syphilitic-node rather than peritoneal T cells, even though both types reduced Mφ functions compared with the activity of the control cells.

Mφ reputedly process antigens by presenting them to lymphocytes for the generation of an appropriate host immune response. Their ability to participate in this activity is closely associated with the expression of an Ia surface antigen (17). The Ia expression in nascent hamster node and peritoneal Mφ was essentially equal, while the differences between these tissue Mφ became noticeable shortly after treponemal infection (Fig. 2). Lymph node Mφ rapidly progressed to express their maximal Ia positivity at 3 weeks postinfection. Conversely, the rate of development of Ia expression by peritoneal Mφ was much slower, and the peak level was significantly less than that demonstrated by node Mφ (Fig. 2). The progression of this activity appeared to be influenced by the presence of the T cell (Table 2). While all nascent T cells appeared to sustain Ia activity of Mφ from treponeme-infected hamsters, only syphilitic-node T cells
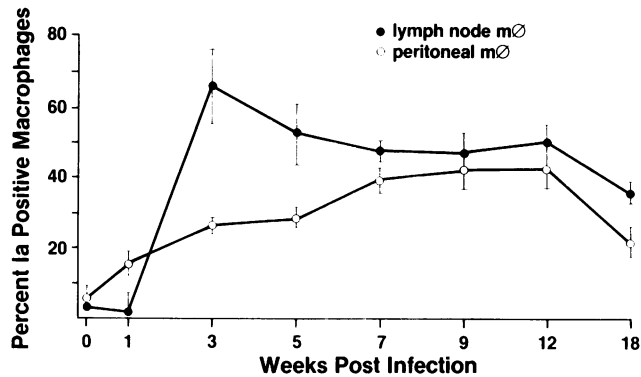
FIG. 2. Expression of Ia activity by Mφ from treponeme-infected hamsters. Mφ from syphilitic animals were assayed by immunofluorescence at selected intervals postinfection. Results represent the mean of experiments performed in triplicate minus background ± SEM.

markedly amplified the expression of this parameter. Moreover, peritoneal T cells from infected animals had a negligible effect on promoting Ia positivity when compared with normal T cells from either source. Therefore, the results suggest that lymph node-derived T cells more readily influence the expression of this response in Mφ from treponeme-infected hamsters than do the peritoneum-derived T cells.

Although maximum suppression of Mφ activity was produced when all cell types were derived from the node, a gradient of these responses could be demonstrated. In this study, regardless of the source of the target Mφ, the syphilitic-lymph-node T cells generated a universally suppressive effect modifying Mφ C3bMI expression. The response was amplified by utilizing mechanisms which recognized and processed treponemal antigen in preference to heterologous antigens (Table 3). This difference was seen by comparing

the magnitudes of the activities produced when either listeria or treponemes were added to the system. Further amplifications of this activity occurred in the presence of live treponemal organisms. The more diminished Mφ reponses suggest that the cells in this operational unit may have the ability to distinguish between live organisms and prepared antigen.

To determine if the observed suppression in syphilitic Mφ could be induced by T cells from sources of perturbation other than syphilitic infection, we performed several coculture studies (Table 4). T cells derived from staphylococcus- or listeria-infected hamsters were shown to have a negligible effect on Mφ alone. However, in the presence of the appropriate homologous antigen, these interactions elicited a response which was consistent with enhanced Mφ activation. In parallel experiments with a nonspecific T-cell activator, concanavalin (ConA), Mφ activity was also generously enhanced. These results confirm that the suppressive effect is confined to a regulatory system characterized by syphilitic T cells and is promoted by the subsequent addition of homologous antigen. In the final set of experiments, we exclusively utilized the syphilitic T cell as a source of regulatory activity to probe the specificity of the system (Table 5). T. pallidum appeared to be more readily distinguished from the other test agents and subsequently induced the suppression of syphilitic Mφ C3bMI well below control levels. In these studies, the heterologous preparations failed to promote similar responses and in a few situations even enhanced the Mφ activity.

DISCUSSION

It has become increasingly apparent that regulation of the cellular host immune responses to treponemal infection is closely associated with the humoral response. Although it is well accepted that substantially effective humoral activity is

TABLE 2. Effect of syphilitic-node and peritoneal T cells on the in vitro expression of syphilitic Mφ Ia positivity

T-cell[a] and Mφ sources[b]	% Ia-positive Mφ[c] after culture for:			
	0 days	2 days	4 days	7 days
Normal node				
Normal	14 ± 3	13 ± 3	17 ± 2	16 ± 3
Infected	58 ± 4	60 ± 2	59 ± 2	59 ± 1
Infected node				
Normal	16 ± 4	24 ± 3	36 ± 2	38 ± 3
Infected	66 ± 3	70 ± 3	68 ± 2	62 ± 4
Normal peritoneum				
Normal	12 ± 4	15 ± 3	14 ± 4	18 ± 2
Infected	63 ± 2	64 ± 3	62 ± 4	60 ± 5
Infected peritoneum				
Normal	14 ± 4	23 ± 3	35 ± 2	33 ± 3
Infected	59 ± 4	59 ± 3	61 ± 3	60 ± 2
No T cells				
Normal	13 ± 2	12 ± 1	7 ± 3	15 ± 2
Infected	55 ± 3	55 ± 2	53 ± 4	53 ± 4

[a] T cells were harvested from lymph nodes and peritonea of normal or infected animals.

[b] Mφ were harvested from nodes of normal or infected animals.

[c] Results after in vitro coculture with T cells, with background values subtracted ± SEM. Experiments were performed in triplicate.

TABLE 3. Introduction of homologous antigen preferentially augments node T-cell regulation of syphilitic Mφ activity

T-cell source[a]	Antigen[b]	Mφ phagocytic response[c]	
		Node	Peritoneum
Node			
Normal	None	51 ± 5	59 ± 4
	List	54 ± 3	57 ± 5
	Tp/−	48 ± 6	54 ± 6
	Tp/+	62 ± 3	68 ± 2
Infected	None	29 ± 3	41 ± 2
	List	49 ± 5	52 ± 3
	Tp/−	18 ± 2	30 ± 4
	Tp/+	9 ± 3	19 ± 5
Peritoneum			
Normal	None	60 ± 3	75 ± 1
	List	66 ± 7	70 ± 4
	Tp/−	56 ± 8	69 ± 5
	Tp/+	66 ± 6	72 ± 7
Infected	None	47 ± 1	61 ± 3
	List	54 ± 5	67 ± 3
	Tp/−	33 ± 2	41 ± 4
	Tp/+	32 ± 4	30 ± 3

[a] T cells were harvested from either normal or treponeme-infected hamsters.

[b] Prepartions of 5 × 10^5 whole-cell formalinized L. monocytogenes (List), formalinized T. pallidum (Tp/−), or live T. pallidum (Tp/+) were added to the in vitro coculture system.

[c] Results are the C3bMI index of experiments performed in triplicate ± SEM.

generated during treponemal infection, migration and eventually widespread dissemination of the organism may still occur in select situations (14, 15, 36).

The organism may avoid total eradication by escaping to an immunologically privileged site within host tissue (43). This observation is confirmed by Sell, who further showed the presence of large numbers of treponemes within nervous tissue (45). Alternatively, other investigations have demonstrated that the in vitro attachment of treponemes to host cells may be prevented by the presence of blocking antibodies produced during infection (18, 38). It is conceivable that similar mechanisms may be operating in vivo to reduce the direct treponeme-cell-mediated interaction and alter the progression of pathogenesis (52).

In the presence of both blocking and opsonizing antibodies, circulating immune complexes may develop (15, 27). Demonstration of the latter species is well documented during secondary syphilitic infection (10, 24, 53). It is these complexes that are subsequently responsible for promoting host autoimmune activity (11). Moreover, immune complexes have also been shown to reduce the immediate availability of complement (9), which is important to the host immune defenses. Circulating complexes may perhaps contribute to the reduction of IgG-mediated ingestion and modulate its formation without blocking IgM (29), which persists in humans infected with treponemes (7). The fact that antibody binding to treponemes is dependent on the presence of complement (37) suggests that utilization of an intact receptor-mediated ingestion system would be a viable option for the cell-mediated response.

However, in this investigation we have shown that the reduction of Mφ activity during infection could significantly contribute to the elusive behavior displayed by the treponeme during its attempt to evade host immune responses. Previously it has been shown that syphilitic T cells are responsible for inhibiting Mφ C3bMI (49), a function that facilitates the eradication of microbes during infection. The

TABLE 5. Specific reduction of Mφ activity by syphilitic-node T cells and a homologous antigen preparation

Antigen source[a]	Phagocytic response[b]
L. monocytogenes	57 ± 2
S. aureus	54 ± 3
ConA	69 ± 2
T. pallidum	28 ± 2
Medium alone	38 ± 2
Mφ alone	52 ± 2

[a] Formalinized organisms (10^6) or ConA (10 μg/ml) was added to coculture systems containing node T cells and Mφ from syphilitic animals.

[b] Node Mφ C3bMI index of experiments performed in triplicate ± SEM.

current work extends these observations by investigating the mechanisms that may be associated with these responses. Regional-node T cells generate a potent Mφ suppression after local intradermal treponemal inoculation. Conversely, intraperitoneal injection of the same preparation fails to promote a similar reduction of Mφ activity (48). These observations suggest that the initial encounter with the organism is perceived differently by the cells contained within each of these individual tissue compartments. This difference implies that each region contains T-cell populations that differ in their orientation and magnitude of regulating the immune response. Studies of lymphocytes from lepromatous and disseminated leprosy confirm that such regional differences can be locally preserved during disease (3, 19). Since the overall percentage of node T cells fluctuates minimally throughout the course of treponemal infection (5), the node may be a stable repository for potent suppressor T cells. Differentiating between the latter and helper T (T_h) cells populations in this tissue during infection will require further examination. However, the data currently available demonstrate that these node T cells best promote suppression of node Mφ. This overall node T-cell response is concordant with the development of pathology at the proximal infection site. This activity would be conducive to a diminished cell-mediated immune response and subsequent establishment of syphilitic lesions in the immediate inguinal region. If this modulation were extended to distal Mφ, similar to the suppressive effect we observed in vitro with peritoneal Mφ, then this mechanism might facilitate the invasion of the treponeme into the host tissues.

This study suggests that T-cell mechanisms are intimately involved in promoting the observed suppressive activity. It is equally important to note that other Mφ functions are not cumulatively inhibited during treponemal infection. Although the C3bMI response is severely diminished, the expression of Ia activity markedly increases and is sustained. This observation implies that a key regulatory circuit operating between the Mφ and T_h cell during antigen presentation remains intact despite the loss of a specific phagocytic response mechanism. Since T_h cells concurrently recruit Mφ and induce their Ia expression, the observed deficit does not appear to be associated with this aspect of the regulatory circuit. Moreover, the rise in host treponemal antibody titer at this stage of infection is consistent with the hypothesis that T_h cells are perceiving antigen as presented by the Mφ. We speculate that smaller, soluble antigen fragments generated by the treponeme facilitate its presentation to T_h cells by Ia-positive Mφ. This approach to promoting antigen recognition avoids processing via the classical route without severely compromising either the presentation of antigen to the T cells or subsequent T_h

TABLE 4. In vitro effect of diverse groups of T cells and antigens on syphilitic Mφ function

T-cell source[a]	Antigen[b]	Phagocytic response[c]
L. monocytogenes	−	69 ± 3
	+	78 ± 2
S. aureus	−	62 ± 3
	+	67 ± 1
T. pallidum	−	36 ± 4
	+	24 ± 3
	+[d]	14 ± 3
ConA	−	66 ± 4
	+	85 ± 2
	+[e]	60 ± 3
Uninfected	−	52 ± 2

[a] T cells were harvested from listeria-, staphylococcus-, or treponeme-infected animal lymph nodes. Additionally, cells were harvested from naive lymph nodes and incubated for 3 days in vitro with ConA (10 μg/ml) before use.

[b] Formalinized organisms (10^6) homologous to the original infection added (+) or no antigen added (−).

[c] Difference between the C3bMI indices for the experimental groups with or without antigen ± SEM. Experiments were performed in triplicate.

[d] Live treponemes (10^5).

[e] 0.1 M α-methylmannoside added to coculture system.

reactivity. There is evidence to show that this is a viable option for the generation of intercellular immune activity (46). This alternate approach may explain why whole, intact treponemes are not readily observed within the Mϕ cytosol. Previously, Lukehart and Miller reported an increased association of treponemal antigens with Mϕ in vitro which progressed with the duration of exposure to the organism (26). Moreover, they observed treponemal "bodies" within the Mϕ cytoplasm. Our results are concordant with these observations. Presentation of treponemal antigens to T cells would require only their close association with the Mϕ surface, although larger antigenic fragments may require Mϕ processing by classical mechanisms which involve some internalization. If one aspect of the Mϕ phagocytic response were altered by suppressor T cells, Fc receptor utilization could partially compensate for the defect despite the loss of C3b receptor activity.

Collectively, the cell responses throughout this study imply that both the induction of T_h activity by antigen-Mϕ complexes as well as the efferent signals emitting from T_h to Mϕ appear to be intact. This possibility suggests that the suppressor T-cell activity may be focused directly on the Mϕ and augmented by T_h enhancement of the suppressor cells. Supplementing this system with homologous antigen would amplify the response, and removing T cells from the environment would diminish the suppression. Both of these activities have been observed throughout this study. However, further investigations will be required to delineate the appropriate sequence(s) of events that yields the observed regulation.

Finally, the magnitude of suppressive activity is elevated when this system is supplemented with live rather than inactivated treponemes. Live organisms have the opportunity to shed antigenic proteins or exocytose soluble molecules or both into the milieu. These entities may be recognized by responsive cells and may subsequently amplify the reaction. Analysis of the homologous antigen studies supports the hypothesis that the mechanism under investigation involves specific recognition at the T-cell level. Alternately, Langerhans cells (16), sinus-lining endothelial cells (30), stimulated fibroblasts (51), and dendritic cells (47) all have the capacity to present antigen to the T-cell fraction. Although the participation of these individual groups is often neglected because of their small quantities (22, 31), their collective contribution may become amplified during infection as Mϕ C3bMI activity functionally diminishes. Thus, an enhancement of this ancillary response may assist the host in sustaining an effective level of antigenic processing. It would be interesting to determine to what extent these populations are utilized. Why the treponeme-host-cell-mediated interaction promotes suppression while other pathogens (e.g., listeria) enhance Mϕ function requires further investigation. Perhaps this activity represents an attempt by the microbe to maintain a stable, symbiotic host-parasite relationship. This rationale also gives credence to the observed concomitant immunoenhancement of the humoral response and suppression of the cell-mediated activity.

ACKNOWLEDGMENTS

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