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Lymphocyte function in experimental endemic syphilis of Syrian hamsters

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Summary. We have studied the changes in the lymph nodes, spleen and thymus that occur in inbred LSH Syrian hamsters infected with *Treponema pallidum* Bosnia A, the causative agent of endemic syphilis, as well as the B-cell responses of these infected animals to helper T-cell independent and dependent antigens. The lymph nodes increased significantly in weight up to 6 weeks after infection, and contained viable treponemes. No significant changes in the spleen weight were observed, and no viable treponemes could be recovered from the spleen. However, the size of the thymus decreased steadily during the course of the disease. The relative number of Ig+ cells (B cells) increased in the spleen and regional lymph nodes, whereas the relative number of T cells decreased during the course of infection. In both the spleen and lymph nodes, the relative number of macrophages increased initially and decreased thereafter in the form of a bell-shaped curve showing a peak at 4–6 weeks of infection. The ability of splenic lymphocytes from infected hamsters to mount a primary PFC response to pneumococcal polysaccharide type III (SIII), a helper T-cell independent antigen, was elevated throughout the course of infection. However, the splenic PFC response to sheep erythrocytes (SRBC), a helper T-cell dependent antigen, was increased only during the first 4 weeks of infection and progressively decreased thereafter. The PFC responses of infected lymph node lymphocytes to both SIII and SRBC were increased during the first 4 weeks and decreased thereafter.

These data suggested that atrophy of the thymus seen in syphilitic infection is accompanied by the complex losses of subsets of T cells and altered B-cell functions. An early loss of suppressor T cells in both the lymph nodes and spleen occurs concomitantly with a loss of T helper cells and heterologous (treponema-unrelated) B-cell functions in the lymph nodes. Helper T cells are lost from the spleen only in the later stages of infection, whereas splenic B-cell functions remain intact throughout the course of the disease. These findings were further tested by *in vitro* methods where splenic and lymph node lymphocytes from infected hamsters were examined for their ability to respond to Con A in terms of the induction of antigen non-specific suppressor T cells. The mixing of Con A stimulated splenic or lymph node lymphocytes from infected hamsters was unable to inhibit the primary antibody responses of SRBC as compared to the normal control. Therefore, the failure of infected splenic and lymph node lymphocytes to induce a normal T-suppressor response may be due to infection-related changes in the subpopulations of T lymphocytes.

Abbreviations: ATS, anti-thymocyte serum; C, complement; Con A, concanavalin A; K, killer cell; NK, natural killer cell; NSE+, non-specific esterase-positive; PFC, plaque-forming cells; SIII, pneumococcal polysaccharide type III; SRBC, sheep red blood cells.

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INTRODUCTION

The immunopathology of syphilis remains poorly understood despite extensive studies performed on humans and experimental animals (Turner & Hollander, 1957; Clark & Danbolt, 1964; Baker-Zander & Sell, 1980; Lukehart, Baker-Zander & Sell, 1980). It is known that a complex host-parasite interaction takes place, but the relative roles of humoral and cellular immunity in the eradication of disease and resistance to reinfection still remain to be elucidated. The existing evidence suggests an important role for both the humoral and the cellular immune response in both human and experimental *Treponema pallidum* infection (Bishop & Miller, 1976; Metzger, Podwinska & Smogor, 1980). Thus, passive immunization of rabbits with hyperimmune rabbit serum obtained during experimental infections delays the onset of the disease and significantly diminishes the extent of the primary lesions (Perine, Weiser & Klebanoff, 1973; Bishop & Miller, 1976). Intradermal injections of immune serum or the IgG fraction of immune serum completely inhibit the development of a chancre in experimental rabbits (Titus & Weiser, 1979). Furthermore, active immunization of rabbits with gamma-irradiated *T. pallidum* protects the animals from initial infection (Miller, 1973). It seems that lymphocytes from outbred rabbits previously exposed to *T. pallidum* and treated with penicillin confer partial resistance onto recipients resulting in fewer lesions and delayed lesions, as compared to unimmunized controls (Metzger *et al.*, 1980). Furthermore, Pavia, Folds & Basemann (1976) have reported that cell-mediated T-cell functions are altered during the earliest stages of *T. pallidum* infection in rabbits.

Recently, we have reported that lymphocytes or T-enriched cell suspensions from hamsters immune to *T. pallidum* Bosnia A strain may passively confer significant protection to uninfected recipients against a challenge by the same strain of treponemes (Schell *et al.*, 1980a, 1981). Since the T cells obviously participate in the immune response to treponemal infection, we wanted to assess the function of various subsets of lymphocytes, correlate the function of T and B lymphocytes, and also establish whether the local immune response parallels the changes observed in the thymus and the spleen. Our data indicate that syphilitic infection leads to thymic atrophy and systemic depletion of T cells. T suppressor cells are affected earlier and to a greater extent than T helper cells and B lymphocytes. Alterations in T helper cell and B-cell functions noticed in local lymph nodes during the course of infection were significantly different from the functional changes of splenic lymphocytes.

MATERIALS AND METHODS

Animals

Male, 10–12-week-old, inbred LSH/Ss Lak strain hamsters were obtained from the Charles River Breeding Laboratories (Willmington, MA). Hamsters weighing 90–110 g were housed six to eight per cage at an ambient temperature of 18°. All the animals were clipped and kept hair-free by weekly clipping before infection (Schell *et al.*, 1980b).

Infection and treponemes

Treponema pallidum Bosnia A strain hamsters were originally obtained from Dr Paul Hardy Jr, Johns Hopkins University, and maintained by passage in LSH hamsters. In order to initiate the infection, the inguinal lymph nodes and cutaneous lesions were removed aseptically, usually from animals infected 5–6 weeks earlier (Schell *et al.*, 1980b; Turner & Hollander, 1957). The skin and lymph nodes were teased apart in sterile saline, and filtered through 60-mesh stainless steel screen. This cell suspension containing viable treponemes was centrifuged at 400 ***g*** for 5 min to remove cellular elements. The supernatant was used to infect the new group of animals. The number of organisms in the supernatant was determined by dark-field microscope. Treponemes (1×10^5) were injected intradermally at the preshaved inguinal regions.

Antigen

Purified pneumococcal polysaccharide type III (SIII, Lot No. L. 667-107-00BD8) was kindly provided by Dr Thomas H. Stoudt (Merck, Sharp and Dohme Institute of Biological Research, Rahway, NJ); immunological properties of SIII from this source have been previously described (Kong & Morse, 1975). Sheep erythrocytes (SRBC) were obtained from Gibco (Grand Island, NY) in Alsever's solutions and were always from the same animal. SRBC were washed three times in sterile saline before use.

Immunization

Antigens were diluted in sterile saline, and the desired dosage (see legends to Tables and Figures) was

injected intraperitoneally in 0·1 ml at various times after infection in hamsters to induce a primary B-cell response.

Assays for antibody activity

For the purpose of demonstrating PFC specific for SIII, target SRBC were coated with SIII antigen according to the previously described technique (Baker, Stashak & Prescott, 1969). Unconjugated SRBC were used as target cells for SRBC-specific PFC, as well as for detecting background PFC responses in animals immunized with SIII antigen.

PFC assays were performed after primary immunization by removing the spleens and inguinal lymph nodes from animals at various stages of infections and from uninfected controls. Single cell suspensions were prepared by gently teasing the organ fragments apart and forcing them through a 1-cm^2 60-mesh stainless steel screen submerged into chilled culture medium (RPMI-1640 containing 10% heat-inactivated fetal calf serum and 50 μg/ml gentamicin). Subsequently, cell clumps and debris were removed by sedimentation at room temperature for 10 min. Cells were washed twice by centrifugation at 400 ***g*** for 5 min in medium before resuspending in serum-free medium for PFC assays.

In order to measure PFC responses to SIII, antigen-coated SRBC were coupled to 60 × 15 mm corning tissue culture dishes with poly-L-lysine (MW 40,000; Sigma, St Louis, MO) according to the technique of Kennedy & Axelrad (1971). Glass tubes (13 × 75 mm) containing 3 ml of serum-free medium, 1 ml of cell suspension ($1 \times 10^{6-7}$ cells/ml) plus 100 μl of guinea-pig serum as a source of C were subsequently plated over the SRBC monolayers and incubated for 45 min at 37°. The PFC responses to SRBC were measured by modified Jerne agarose method (Jerne & Nordin, 1963) in glass tubes (13 × 75 mm) containing 100 μl of cell suspensions ($1 \times 10^{6-7}$ cells/ml). Fifty μl of 20% SRBC suspension and 20 μl of C source, 700 μl of Seaplaque Agarose (Marine Colloids, Rockville, ME) at 47° were added, vortexed and plated over clear, frosted-end, premarked glass slides. After 2–3 min incubation at room temperature necessary for solidification of agarose, slides were incubated in a humidified environment for 4 hr at 37°. Plaques were enumerated with the aid of a dissecting microscope. In the case of SIII-PFC assays, non-specific plaques directed against the indirect cells (unconjugated SRBC) were always subtracted from the total PFC count. In all instances, the background consisted of less than 20 plaques per plate. Only direct (IgM) PFC were counted in the present study.

Microtitre haemagglutination titres against SRBC and SIII-specific serum antibody responses were measured by passive haemagglutination using heat-inactivated (56° for 30 min) sera. Two-fold serial dilutions were carried out in sterile PBS, and an equal volume of 1% suspensions of SRBC (for anti-SRBC antibody titres) and SIII-coated SRBC (for SIII-specific antibody titres) were added to appropriate wells. Plates were covered with lids and incubated in a cold room (4°) overnight. Wells were read for haemagglutination the next morning.

Enumeration of T cells, B cells and macrophages

Cell suspensions (spleen and lymph nodes) prepared from hamsters at various intervals during infection were used in order to enumerate various cell populations in the lymphoid organs in specific stages of infection. B cells (Ig+) were enumerated by standard fluorescent antibody techniques with a polyspecific rabbit anti-hamster Ig serum (Cappel Laboratories, Cochranville, PA) (Raff, 1970). T cells were enumerated with anti-T-cell sera (ATS) and guinea-pig complement as described previously (Blasecki & Houston, 1977; Schell *et al.*, 1980a). Briefly, 200 μl (5×10^7 cell/ml) of cell suspensions were incubated with normal rabbit serum or rabbit ATS. Both sera had been absorbed with SRBC and hamster bone marrow cells (Blasecki & Houston, 1977). After being incubated for 1 hr at 37°, the cell suspensions were washed twice in chilled medium, and the viability of cells in each specimen was enumerated by the eosin-Y exclusion method. Macrophages and monocytes present in the spleen and lymph node cell suspensions were identified by counting the number of non-specific esterase-positive (NSE+) cells (Koski, Poplack & Blaese, 1976). Use of the aforementioned enumeration procedures gave normal values of 25·5+2·1 B cells (Ig+), 61·0+6·8 T cells (ATS+), 2·1+1·2 macrophages (NSE+) and 11·5+3·0 null cells for control inguinal lymph nodes, and 46·5+5·0 B cells, 38·0+4·0 T cells, 4·0+1·0 macrophages and 11·5+3·0 null cells for control spleens.

Cell cultures

Lymphoid cells were cultured in a viable cell density of 1×10^7 cells/ml RPMI-1640 medium, supplemented with 10% fetal calf serum, 100 mM L-glutamine, 25 mM HEPES, 5×10^{-5} M 2-mercaptoethanol and 50 μg/ml gentamicin. One-ml volumes of lymphoid cell suspen-

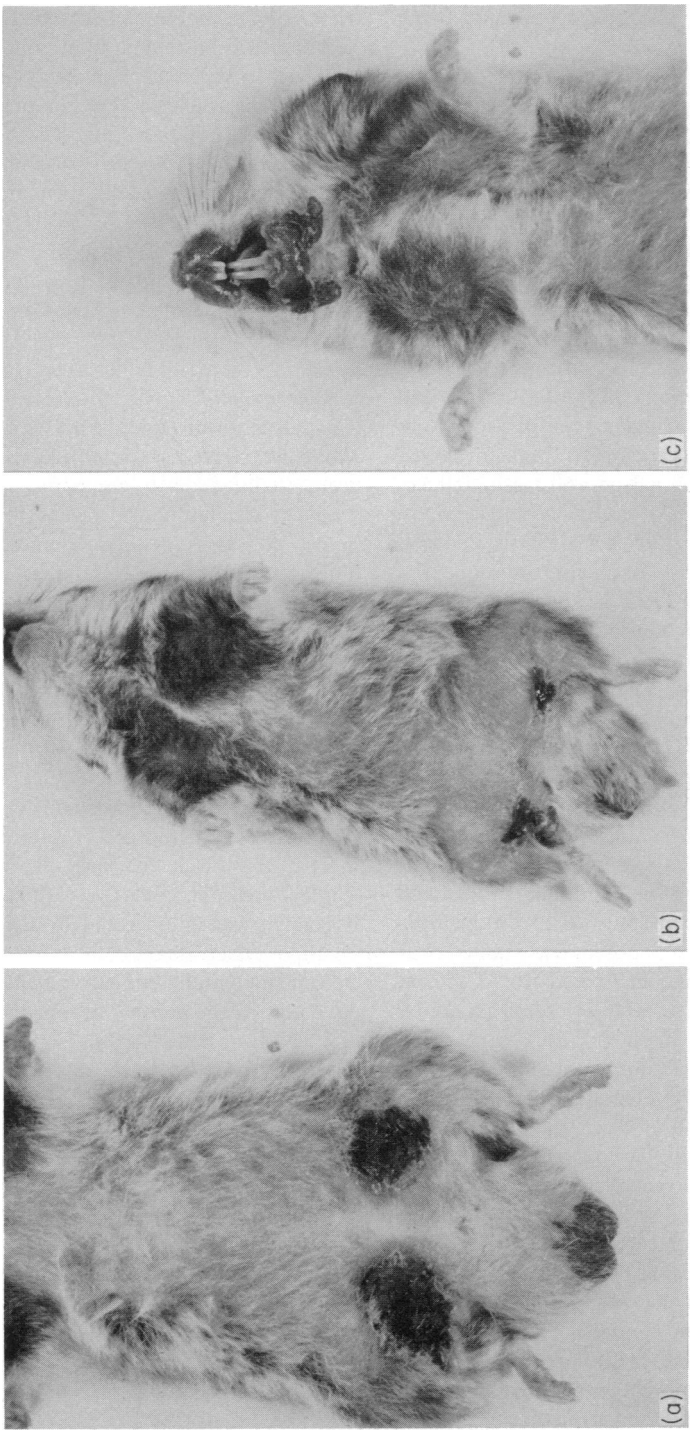

Figure 1. Development, regression and reappearance of secondary syphilitic lesions on the perioral areas: (a) cutaneous lesions at Week 7; (b) primary cutaneous lesions going through a process of healing and small scabs visible at Week 17; (c) appearance of secondary syphilitic lesions, perioral ulcers, on Week 27.

sion (1×10^7/ml) were incubated in a 24-well Falcon plate (Falcon Plastics No. 3047). The immunizing sheep erythrocytes were washed three times with sterile PBS, and 100 μl of 0·5% (v/v) SRBC suspension were added to cells. All plates were incubated for 5 days at 37° in a 6% CO_2/94% air incubator. At the termination of culture period, cells were aspirated from wells of the culture plates into tubes and centrified at 400 *g* for 10 min. Cell pellets were resuspended in 0·1 ml of HBSS, and the number of direct PFCs per culture was assayed as described above. The results are expressed as the number of PFC/10^6 recovered viable cells.

Generation of non-specific suppressor T (Ts) cells

Normal or infected lymphoid cells (1×10^7) were cultured in medium containing 2 mg/ml of Con A. After 48 hr, these Con A-treated cells were harvested, washed three times in sterile serum-free medium, and passively transferred with an equal number of normal spleen or lymph node cultures plus 0·05% (v/v) SRBC. These cultures were then addressed for primary antibody response to SRBC 5 days later. Some of the Con A-treated cells were enriched for T cells by passing them over the nylon wool columns (Schell, 1980a). Control cultures received Con A-stimulated normal lymphoid cells which were pretreated with ATS+C before transfer (Schell, 1980a; Bagasra & Damjanov, 1982) to demonstrate that the transferred suppressor cell is a T-suppressor cell (data not shown).

Analysis of data

Tests for statistical differences in the splenic and lymph node responses of infected *vs* control animals were performed by using the Student's *t*-test. Values for the *t*-statistic were calculated from data derived from the responses of a minimum of six hamsters per experimental variable.

RESULTS

Course of infection

LSH hamsters respond rapidly to infection with *T. pallidum* (Schell *et al.*, 1980b). About 3 weeks after inoculation of *T. pallidum*, all hamsters exhibited erythematous lesions in the inguinal area at the site of injection. The indurated areas enlarged and, at 4 weeks, the skin overlying the induration became ulcerated. Ulceration continued to expand until the sixth to eighth week (Fig. 1a). Thereafter, lesions began to heal slowly, and 16–20 weeks after infection only minor scabs were visible (Fig. 1b). Approximately 24 weeks after infection, the animals spontaneously developed perioral ulcerations which became increasingly severe. Most animals died 28–32

Table 1. Changes in the weight of thymus, inguinal lymph nodes and spleens in hamsters during the course of *T. pallidum* infection

	Inguinal lymph nodes (mg±SEM)	Thymus (mg±SEM)	Spleen (mg±SEM)
Control I (uninfected) (15 weeks)	23±2·9	117·3±6·7	224·6±8·1
Infected			
1 week	29·3±3	83·5±12*	219·3±6
2 weeks	36·8±4*	62·1±6†	223·8±9
4 weeks	72·1±6†	50·9±8†	233·4±7
6 weeks	100·2±10†	40·0±6†	231·1±9
8 weeks	102±12†	35·6±5†	245·0±5
6 months	31±3	23·0±7†	241·8±10
Age matched uninfected Control II (6 months)		82·6±11*	

Six hamsters per group were used to calculate the mean±SEM. Uninfected pristine animals were used as control.

* $P < 0·05$ (*vs* Control I).

† $P < 0·001$ (*vs* Control I).

Table 2. Changes in the relative number of macrophages, T lymphocytes, B lymphocytes and null cell populations in inguinal lymph nodes during the course of *T. pallidum* infection in LSH Syrian hamsters

	Percentage of cells ± SEM			
	Macrophages	T lymphocytes	B lymphocytes	Null cells*
Normal (Control)	2·1 ± 1·2	61·0 ± 6·8	25·5 ± 2·1	11·5 ± 3
Infected§				
1 week	2·9 ± 1	22·0 ± 1‡	27·0 ± 6	48·1 ± 3§
2 weeks	4·2 ± 1	25·5 ± 2‡	31·0 ± 2§	39·3 ± 1§
4 weeks	4·6 ± 1	29·0 ± 4‡	35·5 ± 3§	30·9 ± 3§
6 weeks	4·2 ± 1	31·0 ± 6‡	37·5 ± 2§	27·3 ± 3‡
8 weeks	3·8 ± 1	35·5 ± 4‡	38·0 ± 2§	22·7 ± 3†
6 months	3·5 ± 1	33·0 ± 3‡	33·0 ± 2§	30·5 ± 2‡

Six hamsters per group were used to calculate the mean percentage ± SE.
* Null cells were calculated according to the following formula:

$$\% \text{ null cells} = 100 - (\%\text{T cells} + \%\text{ B cells} + \%\text{ MØ}).$$

† $P < 0{\cdot}05$ (*vs* controls).
‡ $P < 0{\cdot}01$ in this column (*vs* controls).
§ $P < 0{\cdot}001$ (*vs* controls).

weeks after infection due to malnutrition (Fig. 1c). The animals were unable to eat due to perioral lesions.

Anatomically, the only remarkable changes were seen in lymph nodes and the thymus. The weight of inguinal lymph nodes increased progressively up to 6–8 weeks after infection and decreased to normal levels thereafter (Table 1). The weight of the thymus decreased rapidly during the first week and steadily thereafter (Table 1). No significant changes in the weight of the spleen were noticed throughout the course of the disease.

Cellular changes in lymphoid organs of infected hamsters

As shown in Table 2, the enlargement of the inguinal lymph nodes characterizing the early stages of the disease is accompanied by a slight increase in the relative number of macrophages, B lymphocytes and null cells, and a substantial decrease in the relative number of T lymphocytes. An increase in the relative number of B lymphocytes became evident at 4 weeks postinfection and persisted thereafter. The relative number of T lymphocytes decreased during the first week postinfection and remained essentially unchanged throughout the course of the disease. The number of cells that did not have the markers of B or T lymphocytes or macrophages (termed 'null cells') increased dramatically as early as 1 week after infection and remained high throughout the course of the disease.

One week postinfection, the spleens of infected hamsters contained the same relative number of macrophages and B cells as the control animals, but at the same time exhibited a significant decrease in the relative number of T lymphocytes, coupled with an increased number of null cells (Table 3). The relative number of macrophages in the spleen increased slightly thereafter, peaked at 4 weeks, and declined to normal levels after the eighth week postinfection. The relative number of T lymphocytes remained low throughout the course of the disease. The relative number of B lymphocytes increased at 4 weeks after infection and remained high thereafter. An increased number of null cells was noticed only during the first 2 weeks postinfection. At 6 months postinfection, the number of null cells was lower than in control animals.

PFC dose response to helper T-cell independent and dependent antigens

A pilot programme was carried out to determine the optimal dose of SIII and SRBC for the PFC test in LSH hamsters. The optimal dose of SIII was 0·5 μg per hamster (data not shown). This dose of antigen injected intraperitoneally produces a maximal PFC

Table 3. Changes in the relative number of splenic macrophages, T lymphocytes, B lymphocytes and null cell populations during the course of *T. pallidum* infection in LSH Syrian hamsters

	Percentage of splenic cells (±SEM)			
	Macrophages	T lymphocytes	B lymphocytes	Null cells*
Normal (Control)	4·0±1	38·0±4	46·5±5	11·5±3
Infected§				
1 week	4·2±1	24·0±1‡	43·0±4	28·8±2¶
2 weeks	6·4±2†	22·0±3	48·7±8	22·9±4¶
4 weeks	8·4±1†	21·5±2	56·0±4§	14·1±4
6 weeks	8·0±2†	18·0±2	58·0±3§	16·0±2
8 weeks	5·8±1†	19·5±4	62·0±6§	12·7±4
6 months	5·2±1†	25·0±3	67·0±4§	2·8±3

Six hamsters per group were used to calculate the mean percentage ± SEM.
* Null cells were calculated according to the following formula:

$$\% \text{ null cells} = (\% \text{ T cells} + \% \text{ B cells} + \% \text{ MØ}) - 100.$$

† $P < 0{\cdot}05$ (*vs* controls).
‡ $P < 0{\cdot}01$ for all values in this column *vs* controls.
§ $P < 0{\cdot}05$ (*vs* controls).
¶ $P < 0{\cdot}001$ (*vs* controls).

response in the spleen and the inguinal lymph nodes both in the infected and uninfected hamsters. In infected animals, the PFC response was more pronounced than in the uninfected animals. In both groups of animals, the numbers of PFC were higher in lymph nodes than in the spleen. The dose of SRBC required for the maximal PFC response was different for the spleen than the lymph node. Thus, the optimal dose was 5×10^8 SRBC for the splenic PFC and 1×10^8 for the lymph node PFC. The infected animals showed a higher PFC response than the uninfected animals, but the optimal dose producing the maximum PFC response was the same in both groups.

PFC response to helper T-cell dependent and independent antigens at various time intervals following immunization

Using the optimal dose of SIII (0·5 μg/hamster) and 3×10^8 SRBC (a dose corresponding approximately to

Table 4. Primary PFC responses in spleen and lymph nodes of Syrian hamsters at various intervals after immunization with SIII and SRBC

Source of lymphocytes	Day 4	Day 5	Day 6	Day 7	Day 8
SIII-specific PFC/10^6 cells* (0·5 μg/hamsters)					
Spleen	161±22	421±14	393±41	120±20	17±5
Lymph node†	255±41	635±43	620±58	169±29	31±9
SRBC-specific PFC/10^6 cells† (3×10^8 cells)					
Spleen	613±16	4037±114	1139±158	460±29	87±19
Lymph node†	168±28	306±11	303±31	105±13	45±12

* Mean responses are the direct PFC obtained from six normal hamsters per experimental variable.
† Inguinal lymph nodes were used for studies.

the mean of the doses producing the maximal PFC response in the spleen and the lymph node), it was determined that a maximal PFC response was obtained on Day 5 postimmunization (Table 4). PFC response to SRBC was more pronounced in the spleen than in the lymph nodes, whereas the opposite was true for SIII.

Antibody response to the helper T-independent antigen during the course of the disease

PFC response to antigens such as SIII in helper T-cell independent and can provide a quantitative measure of intrinsic B-cell function (Baker *et al.*, 1973). In the present study, hamsters were immunized with SIII at various times during infection with *T. pallidum*, and the magnitude of the subsequent primary antibody response was determined. The infected animals showed a marked increase in the PFC response to SIII as early as 1 week postinfection (Fig. 2). The splenic PFC remained high throughout the course of the disease. In the lymph nodes, an increased PFC was seen only during the first 4 weeks postinfection. After the sixth week postinfection, a significant suppression of the PFC was noticed in the lymph nodes. SIII-specific serum antibody responses monitored by passive haemagglutination tests with SIII-coated SRBC paralleled the PFC response (Fig. 2).

Antibody response to helper T-dependent antigen during the course of the disease

An increased PFC response to SRBC was noticed in the spleen and the lymph nodes during the first 4 weeks

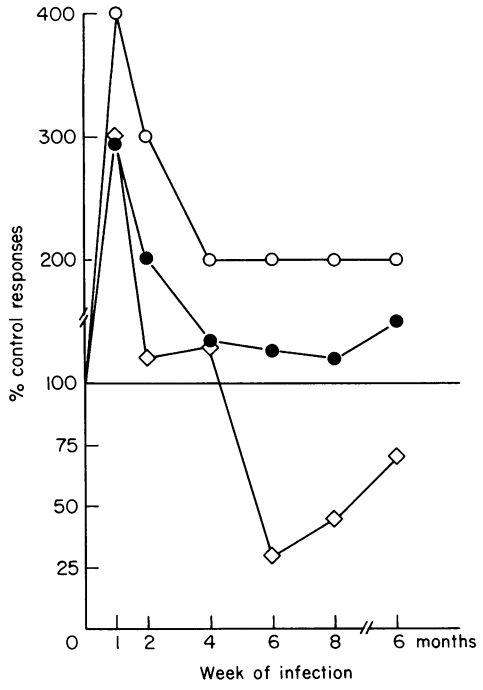

Figure 2. Primary splenic (●——●) and inguinal lymph node (◊——◊) PFC, and serum antibody (○——○) responses of *T. pallidum* Bosnia A strain-infected LSH inbred hamsters to SIII antigen. At the time of infection, separated groups of age-matched hamsters were immunized with SIII (0·5 μg), and primary splenic and lymph node PFC and serum antibody responses to the antigen were measured 5 days later. Mean IgM PFC control counts are shown on a PFC/10^6 cell basis; serum antibody titres were converted to $\log_2$ values for comparison. Results are those of a representative experiment.

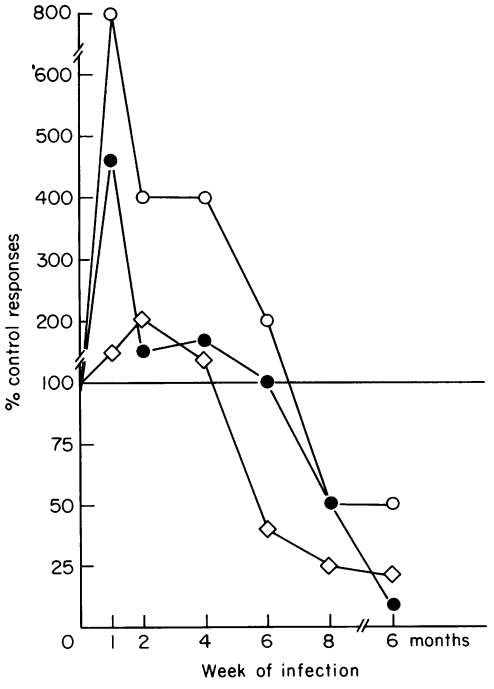

Figure 3. Primary splenic (●——●) and inguinal lymph node (◊——◊) PFC, and serum antibody (○——○) responses of *T. Pallidum* Bosnia A strain-infected LSH inbred hamsters to SRBC. At the time of infection, as shown, separate groups of age-matched hamsters were immunized with SRBC (3×10^8 SRBC), and primary splenic and lymph node PFC as well as serum antibody responses to the antigens were measured 5 days later. Mean IgM PFC control is shown on a PFC/10^6 cell basis; serum antibody titres were converted to $\log_2$ values for comparison. Results are those of a representative experiment.

Table 5. Primary PFC responses to SRBC and effect of Con A-stimulated lymphocytes

Normal* cell culture	Con A-stimulated† cells added	PFC/10^6 cells	% inhibition
(1) Spleen cells	None	86±17	–
		56±12	–
(2) Lymph node cells	None	1289±101	–
		343±49	–
(3) Lymph node cells	Normal spleen	12±2	86%
		16±3	71%
(4) Lymph node cells	Normal lymph node cells	26±7	98%
		17±13	95%
(5) Lymph node cells	Infected spleen cells (4 weeks)	1091±61	15%
		421±29	−23%
(6) Lymph node cells	Infected lymph node cells (4 weeks)	1259±112	3%
		289±41	15%
(7) Lymph node cells	Infected spleen cells (8 weeks)	1420±218	−10%
		516±62	−50%
(8) Lymph node cells	Infected lymph node cells (8 weeks)	996±87	23%
		232±71	32%

* Normal splenocytes or lymph node cells were cultured with SRBC (0·05%) for primary plaque-forming cell response *in vitro*.

† 1×10^7 cells were cultured for 48 hr with 2 mg of Con A, washed and added to an equal number of normal cells plus SRBC and addressed for their ability to inhibit the primary PFC response *in vitro*.

postinfection (Fig. 3). However, after the initial 4-week interval, the PFC response of both splenic and lymph node cells decreased below the controls of the disease.

Evaluation of antigen non-specific suppressor T-cell (Ts) activity

Although B-cell response to SIII is not dependent upon helper T cells for induction, the magnitude of the developing response is regulated by other T-cell subpopulations such as suppressor T cells (Kennedy & Axelrad, 1971; Kerbel & Eidinger, 1972). Since the proportion of T cells decreases in lymph node and spleen (Tables 2 and 3) during infection, and since early splenic and lymph node responses to SIII and SRBC are enhanced, we asked whether the enhanced response to SIII and SRBC observed during *T. pallidum* injection could be attributed to an inability of suppressor T cells to regulate appropriately. This could be a result of a preferential dysfunction or decrease in the T-suppressor cell populations. We therefore attempted to evaluate the antigen non-specific suppressor T-cell activity in the lymphoid populations of spleen and lymph nodes by activating the T-suppressor cell populations with mitogenic concentrations of Con A (Tadakuma & Pierce, 1978). Con A-stimulated lymphoid populations (1–2 mg Con A/10^7 cells for 48 hr) have been shown to suppress PFC responses to SRBC non-specifically by murine spleen cells *in vitro* (Tadakuma & Pierce, 1978).

As shown in Table 5, the normal splenic and lymph node primary anti-SRBC PFC responses are significantly inhibited by the addition of corresponding Con A-stimulated normal lymphoid cells (71–95% inhibition). However, when splenocytes from infected hamsters were examined for their non-specific suppressor T-cell function, they exhibited a significantly less suppressive activity compared with their normal counterparts. Therefore, splenocytes from 4 weeks postinfection were able to suppress the primary (IgM) PEC response to SRBC only up to 15% in one experiment, and PFC response was 123% enhanced in another experiment. Similarly, splenocytes at 8 weeks postinfection exhibited a lack of inhibitory activity, and PFC responses in both experiments were

enhanced. Lymph node cells from both 4- and 8-week postinfection hamsters were found to express an inhibitory activity but, in all four, experiments were significantly depressed as compared to controls.

DISCUSSION

The immunocompetence of hosts infected with *venereal pallidum* has been the subject of active research for several years. The general belief for some time was that the host immune system is depressed during syphilitic infection (Griedmann & Tur, 1975; Pavia *et al.*, 1976; Wicher & Wicher, 1977a, b; Baughn & Musher, 1978). This concept probably originated and was based on findings in humans. In man, the delayed type hypersensitivity reactions to treponemal antigens (Noguchi, 1911; Csonka, 1950; Marshak & Rothman, 1950), resistance to various infections (Fiumara & Lessell, 1970) and leucocyte migration inhibition (Fulford & Brostoff, 1972; Wicher & Wicher, 1975) were found to be depressed. Histological examination of the paracortical areas of lymph nodes of patients with early syphilis reveal both lymphocyte depletion and histiocytic proliferation (Turner & Hollander, 1957; Baker-Zander & Sell, 1980). Spleens of congenitally syphilitic infants (Levene, Wright & Turk, 1971) and neonatally infected rabbits also exhibit similar changes (Festenstein, Abrahams & Bottenheuser, 1967). However, the experimental data obtained in animals do not support the hypothesis on depressed cell-mediated immunity in syphilis (Lukehart *et al.*, 1980; Metzger *et al.*, 1980).

An important step forward in the study of experimental treponemal infections has been made with the introduction of a new animal model—the inbred LSH Syrian hamsters (Schell *et al.*, 1980a, b, 1981). Turner & Hollander (1957) initially explored the susceptibility of hamsters to *T. pallidum* Bosnia A strain. LSH hamsters injected intradermally with *T. pallidum* Bosnia A strain mimic the primary form of syphilitic lesions in humans (Schell *et al.*, 1980a, b, 1981).

Using the inbred hamster model of experimental syphilis, we have now shown the following: (i) the immune response in early stages of endemic syphilitic infection differs considerably from the response in later stages of the disease; (ii) local changes in the lymph nodes adjacent to the lesion differ from the changes in the central lymphoid organs (spleens and thymus); (iii) thymic atrophy which starts early in the disease progresses in later stages and could account for many of the immune dysfunctions observed. These changes are, however, extremely complex and it is not possible to conclude to what extent they correspond to the changes seen in humans.

If one looked only at the size and weight of various lymphoid organs, one could have concluded that changes induced by *T. pallidum* infection of LSH hamsters occur only in the thymus and the local lymph nodes. The thymus undergoes atrophy and the lymph nodes enlarge initially and then return to normal size. The weight of the spleen does not change during the course of the infection. However, analysis of the various cell populations in the spleen revealed that splenic changes are as dramatic (although qualitatively different) as the changes in the thymus and lymph nodes. This suggests that the entire immune system of the body reacts to *T. pallidum* infection. Microbes were, however, isolated only from the skin lesions and the local lymph nodes. This, in turn, may signify that the activation of the systemic immune response is most probably due to the release of the treponemal antigens into the circulation, or due to the cellular transmission of antigens by the locally exposed cells. The increased number of macrophages in the lymph node and the spleen favours the latter hypothesis. In a previous paper (Bagasra & Damjanov, 1982), we have shown that *T. pallidum* infection indeed activates macrophages, and that the macrophages are able to process and present the microbial antigens to T lymphocytes throughout the course of the disease.

The cellular changes in the local lymph nodes could be divided into three distinct phases. In the first phase (roughly corresponding to 2 weeks), a marked decrease of T cells has been noticed, accompanied by a marked increase in the null cell population. The second phase was characterized by an increase of B cells and macrophages. This phase lasted approximately through the sixth week postinfection. The third phase was accompanied by the reduction of the weight of the lymph nodes to preinfection levels and was coincident with the disappearance of *T. pallidum* from the organism. It was characterized by persistently high percentages of B lymphocytes and null cells and lower percentages of T lymphocytes. The initial enlargement of lymph nodes was mostly due to the increased number of null cells. The exact nature of these cells is not known. Theoretically, it is possible that these cells represent precursors of mature T and B lymphocytes, K or NK cells, mature lymphoid cells which have lost their surface markers, or non-lymphoid cells.

The thymus of infected animals underwent severe atrophy, beginning with a rapid decrease in weight

during the first week, followed by a less pronounced but still persistent involution thereafter. The loss of thymic tissue correlated with the loss of T cells in the spleen and the local lymph nodes. Our *in vivo* studies with T-dependent and independent antigens, as well as our *in vitro* studies showing unresponsiveness to Con A stimulation in terms of the induction of antigen non-specific suppressor T cells, suggest that suppressor cells are lost earlier and at a higher rate than T helper cells. This conclusion was derived from the *in vivo* data showing an initially increased PFC response to SRBC and SIII antigens (Figs 2 and 3), i.e. loss of suppressor T-cell activity on the B lymphocytes (Baker *et al.*, 1969, 1973, 1974). In mice as well as Syrian hamsters (our unpublished observations), the elimination of suppressor T cells results in a greatly enhanced primary PFC response SIII and other T-cell independent antigens when compared to T-cell intact animals (Baker *et al.*, 1969, 1973, 1974; Kerbel & Eidinger, 1972). The elevated PFC responses to SIII in spleen and in lymph nodes up to within 4 weeks of infection could thus be the result of the dilution or relative dysfunction of suppressor T-cell subpopulations. There are, however, other possible explanations for the elevated primary B-cell responses to SIII. For example, there may be non-specific activation of B lymphocytes due to some product(s) or component of treponemes. Therefore, in order to further evaluate the suppressor T-cell function in splenic and lymph node populations in *T. pallidum*-infected hamsters, we performed *in vitro* cell mixture experiments. The lymphocytes from infected hamsters were examined for their ability to suppress the primary antibody respone to SRBC *in vitro* after 48 hr mitogenic activation by Con A (Tadakuma & Pierce, 1978). Both splenic and lymph node population from 4-week and 8-week postinfection animals exhibited a significant ($P < 0{\cdot}001$) responsiveness to Con A stimulation in terms of *mitogenic* induction of antigen non-specific suppressor T cells as compared to normal controls, indicating an intrinsic loss of suppressor T-cell responsiveness in these populations. In three out of four experiments, the splenic lymphocytes from 4-week and 8-week postinfected animals exhibited an enhancement effect on primary *in vitro* antibody response. These could simply be the result of transfer of higher B-cell populations present in splenic lymphocytes from infected animals. The lymph node lymphocytes from infected animals also seem to have lost the intrinsic suppressor T-cell activity, but to a lesser extent. In 8-week postinfected lymph nodes there was an increase in the suppressor T-cell activity, as compared to the 4-week postinfection group, indicating a recovery of this T-cell subset. This could explain why both anti-SIII and SRBC PFC responses are decreased after 4 weeks postinfection. However, further studies are needed to clarify this statement.

Helper T-cell antigen responses in spleen and lymph nodes were also found to be enhanced up to 4 weeks after infection, and were significantly depressed thereafter. The degree of unresponsiveness became more pronounced during the course of infection. Our findings are essentially in agreement with previous studies concerning primary *in vivo* early antibody responses to SRBC in rabbits infected with *T. pallidum* (Baughn & Musher, 1978). In the present study, the inability of splenic B cells to respond to helper T-cell dependent antigen was probably due to a defect at the level of the T helper cell rather than B cell, since splenic intrinsic B-cell responses to SIII were intact throughout the course of infection (Baker *et al.*, 1969, 1973; Kerbel & Eidinger, 1972).

In the present study, we have not explored the treponema-associated mechanisms responsible for lymphocyte dysfunction in the course of the disease. Several different mechanisms might contribute to the observed immunoenhancement and suppressions. For example, Basemen & Hayes (1980) have described receptor-binding proteins for *T. pallidum* which could influence the entry of the microbe into the macrophages or their interaction with lymphocytes. It is possible that these proteins may bind more avidly to one or more lymphocyte populations, and passively or actively interfere with their functions. The existence of T-cell dysfunction has been suggested by earlier studies showing abnormal lymphocyte response *in vitro* (Griedmann & Tur, 1975; Pavia, Folds & Basemann, 1977; Wicher & Wicher, 1977a, b; Metzger *et al.*, 1980; Lukehart *et al.*, 1981). In other studies mucopolysaccharide (Bey *et al.*, 1979) or serum or plasma factor(s) present in patients with secondary syphilis (Lukehart *et al.*, 1981) or in experimentally infected rabbits (Wicher & Wicher, 1977b; Maret, Basemann & Folds, 1980) have been identified. Circulating immune complexes have also been postulated as a cause of depressed lymphocyte functions (Baughn, Tung & Musher, 1980), since patients with secondary syphilis exhibit elevated levels of circulating immune complexes, and immune complexes bound to T lymphocytes suppress the differentiation and proliferation of B cells in the presence of mitogens (Finbloom *et al.*, 1981).

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