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T-Cell-Independent Elimination of *Borrelia turicatae*

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Mice deficient or deprived of thymus-derived lymphocytes eliminated blood-borne *Borrelia turicatae* with efficiency comparable to that observed in normal littermates. When challenged with 10^5 borreliae, nude mice had mean (± standard deviation) primary spirochetemias lasting 3.1 ± 0.2 days and mean (± standard deviation) peak bacterial counts of $3.0 \times 10^7 \pm 0.5 \times 10^7$ cells per ml of blood; in comparison, heterozygous littermates and normal mice had respective primary spirochetemias lasting 3.4 ± 0.6 and 3.2 ± 0.2 days and respective peak counts of $8.0 \times 10^7 \pm 1.5 \times 10^7$ and $5.5 \times 10^7 \pm 0.9 \times 10^7$ bacterial cells per ml of blood. No increased responsiveness to concanavalin A was observed in infected nude mice, indicating the sustained lack of maturate T cells in these animals. Thymectomized and steroid-treated mice were also found to eliminate circulating borreliae with efficiency comparable to that observed in control animals. Irradiation of mice abrogated responsiveness to borreliae, but reconstitution with T-cell-depleted splenocytes restored antibody production. It is proposed that elimination of *B. turicatae* is mediated by a T-cell-independent immune response mechanism.

The underlying mechanism for immune elimination of blood-borne borreliae that cause relapsing fever has remained poorly defined for nearly three-quarters of a century. Studies have revealed that passive administration of immune serum could delay the onset of or afford complete protection against experimental borrelemia in laboratory animals (2, 8, 17). Immunoglobulins M and G have both been shown to afford passive protection against *Borrelia* spp. (2, 8). As immunoglobulin M is a potent complement-fixing immunoglobulin (21, 25) and is not directly opsonic, it has been suggested that complement-mediated lysis may be an important mechanism for the elimination of borreliae (12). It has recently been demonstrated, however, that an intact lytic complement pathway is dispensable, as C5-deficient mice can effectively eliminate *Borrelia turicatae* (24).

The contribution of lymphocytes toward the development of a protective immune response to *Borrelia* spp. has received little attention in the past. It has been documented that peripheral blood lymphocyte counts generally increase during spirochetemia (6, 12). Wright (27) has reported that the immunosuppressive drug cyclophosphamide prevents antibody formation and thus abrogates the elimination of borreliae. Yet, the collective role of mammalian T and B lymphocytes in response to borreliae is unclear. This paper presents findings from in vivo studies aimed at assessing the need for T lymphocytes in the elimination of *B. turicatae*.

MATERIALS AND METHODS

Animals. BALB/c mice were obtained from the mouse colony of D. W. Watson, University of Minnesota, Minneapolis. BALB/c-derived homozygous and heterozygous mice carrying the nude trait were obtained from Taconic Farms, Inc., Germantown, N.Y. AKR/J mice were obtained from Jackson Laboratories, Bar Harbor, Maine. Homozygous and heterozygous mice carrying the nude trait were housed in cages containing autoclaved food and bedding and fitted with a dust filter. All other mice were housed in groups of five per cage and provided with food and water ad libitum.

Bacteria. *B. turicatae* M2007AN129 was a homogeneous phase variant. Organisms were enumerated as described elsewhere (24).

Immunosuppression of mice. Mice received 750 rads of whole-body irradiation (X ray) or 300 mg of cyclophosphamide (Mead Johnson & Co., Evansville, Ind.) per kg of body weight 1 day before being used experimentally.

Surgical ablation of thymus. Newborn SW mice were obtained within 3 h of birth. The method of Castro (9) was followed for neonatal thymectomy. Each newborn mouse was chilled on ice in lieu of chemical anaesthesia. When body movement was substantially retarded, the limbs and head of the animal were firmly fastened to a stiff board with elastic straps. With the assistance of a dissecting microscope, a sagittal incision was made across the clavicle. The thymic lobes were exposed by reflection of the incised tissue and removed with gentle suction. Sham control animals underwent the identical procedure with the exception of thymic removal. A figure-eight stitch was sewn with Ethicon no. 6 catgut suture to close the wound. Each animal was then warmed under a 150-W infrared lamp (ca. 35°C) for 1 h before being returned to the parent. Short-term mortality averaged 30% for thymectomized animals and 0% for sham controls. Animals were maintained under clean conditions until use.

Lymphocyte transformation assay. Animals were sacrificed by asphyxiation with chloroform. The left side of each animal was disinfected with 70% ethanol in water. A vertical incision was made approximately 3 mm caudal from the rib cage and extended toward the spine. The spleen thus exposed was removed with the aid of sterile forceps and transferred to a petri dish containing RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.). Each spleen was split laterally, and splenocytes were freed by directing a stream of culture medium onto the organ with a syringe fitted with a 25-gauge needle. The resulting cell suspension was harvested and washed once with fresh RPMI 1640 medium, and erythrocytes were lysed as described by Boyle (3) and subsequently layered onto a Ficoll-Hypaque cushion. Cells were subjected to centrifugation for 15 min at 1,500 × *g* in a Beckman TJ-6R benchtop centrifuge. Lymphocytes found at the medium-Ficoll interface were harvested, washed, and resuspended in RPMI 1640 medium–10% fetal bovine serum to a final density of 2.0×10^7 cells per ml. Cell viability, as

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ascertained by trypan blue exclusion, was greater than 95%. A 50-μl inoculation of splenocyte culture was transferred to each of 12 microculture wells and tested for responsiveness to mitogens as described by Waithe and Hirschhorn (26). Serial dilutions of concanavalin A (Sigma Chemical Co., St. Louis, Mo.) and lipopolysaccharide B (Difco Laboratories, Detroit, Mich.) at respective concentrations of 10, 2, or 1 and 200, 20, or 2 μg were added to cells 2 or 3 days before labeling; one set of cells was left untreated to serve as controls. All samples were prepared in triplicate. The cells were labeled with 50 μCi of [^{3}H]thymidine and harvested 6 h later with a cell harvester-washer apparatus (Otto Hiller Co., Madison, Wis.). The specimens were dried under a heat lamp for 30 min. Radiolabel was detected by placing dry specimens into 1.5 ml of aquasol and counting in a Beckman LS-100C scintillation counter.

Adoptive lymphocyte transfer. The method of Sherr et al. (25) was used to reconstitute AKR/J mice with selected populations of lymphocytes. Briefly, 10-week-old mice that hhad been deprived of food for 24 h were exposed to 750 rads of whole-body irradiation at 4 h before cell transfer. Six-week-old donor mice were sacrificed by CO_2-induced hypoxia, and spleens were aseptically removed. Splenocytes were gently teased from the organs by directing a stream of prewarmed (37°C) Hanks balanced salt solution into spleens with a tuberculin syringe and needle. The cells were washed once and then treated with either anti-immunoglobulin (Cappel Laboratories, Cochranville, Pa.) or anti-thy 1.1 produced in C3H/HeJ mice. After 15 min, the sensitized cells were treated with guinea pig complement; 47% of splenocytes treated with anti-immunoglobulin and 39% of splenocytes treated with anti-thy 1.1 were lysed by complement, as determined by trypan blue exclusion assay. The cells were washed twice in Hanks balanced salt solution, and 2.5×10^7 cells were injected intravenously into irradiated recipients. Recipients of T plus B cells were given 2.5×10^7 each of the donor splenocyte population (total of 5.0×10^7 cells). Mice were used 1 week after reconstitution.

Fluorescent-antibody staining. Fluorescent-antibody staining was performed on glass (Fluoroslide)-fixed blood. Briefly, serum diluted 1:10 in saline was allowed to adsorb to specimens for 15 min in a humid chamber. (This step was omitted when we were attempting to detect preattached serum components.) Unbound antibodies were removed by several 5-min washings in phosphate-buffered saline. A 1:10 dilution of commercially prepared fluoresceinated antiserum against the serum component investigated was applied to the specimen, and incubation was allowed to proceed for an additional 30 min. The slides were removed from the humid chamber and rinsed in phosphate-buffered saline for 15 min to remove unbound antibodies. Phosphate-buffered 90% glycerol was applied to the specimen and covered with a cover slip. The specimens were viewed under a Leitz H_2 deuterium-lamp UV microscope.

RESULTS

The variant antigen against which the antibody response is mounted has been identified as proteinaceous in nature. Of interest are the reports that immunity against given variants of borreliae is short lived (10, 13, 14). It was thus of value to evaluate the specific lymphocyte requirement for elimination of *B. turicatae* to better understand the observed antibody response to these organisms.

Irradiation of mice was found to prevent both elimination of borreliae (Fig. 1) as well as production of detectable antibody as determined by fluorescent-antibody staining;

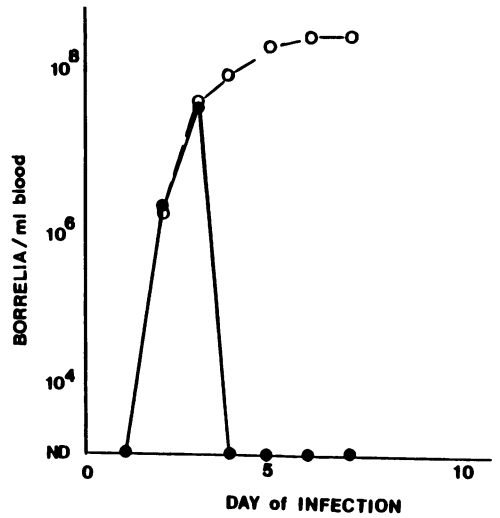

FIG. 1. Effect of irradiation on ability of the host to eliminate borreliae. Symbols: ●, nonirradiated controls; ○, irradiated mice.

corresponding control animals eliminated borreliae and had detectable antibody as demonstrated by fluorescent-antibody staining (data not shown). Similarly, the immunosuppressive drug cyclophosphamide was found to effectively abrogate the elimination capacity against borreliae when administered at a dose of 300 mg/kg of animal weight (Fig. 2). The ability to eliminate spirochetes was not regained during the course of these 2-week studies.

Because immunosuppression by radiation or radiometric drugs acts nonspecifically and such therapy affects cell types other than lymphocytes (e.g., polymorphonuclear leukocytes), it was necessary to more specifically define the role of distinct lymphocyte populations in the elimination response. The role of the T lymphocyte population was first examined in mice thymectomized within 12 h of birth. It was found that the pattern of spirochetemia in infected, thymectomized mice did not differ appreciably from that or corresponding sham controls, with respective first peaks (mean ± standard deviation) of $8.5 \times 10^7 \pm 4.0 \times 10^6$ and $8.5 \times 10^7 \pm 3.5 \times 10^6$ and respective duration periods of 3.9 ± 0.6 and 3.7 ± 0.4 days (Fig. 3).

Congenital T-cell-defective nude mice gave results similar to those observed with surgically thymectomized mice (Fig. 4). The homozygous nude mice had borrelemias of $3.0 \times 10^7 \pm 5.0 \times 10^6$ cells per ml of blood, with a mean first-attack duration of 3.1 ± 0.2 days. The heterozygous nude and homozygous control animals gave respective peak counts of $8.0 \times 10^7 \pm 1.5 \times 10^7$ and $5.5 \times 10^7 \times 0.9 \times 10^6$ cells per ml of blood.

Because it has been documented that certain bacterial and viral agents can induce maturation of precursor T cells in nude mice (22), it was essential that mice be screened for the presence of matured T lymphocytes immediately after cessation of the initial borrelemia. Total splenocytes were screened for responsiveness to concanavalin A as a marker of T cell maturity (22). Although heterozygous infected mice incorporated up to 6,527 ± 612 cpm of [^{3}H]thymidine after optimal concanavalin A stimulation for 3 days at 1 μg per culture (Table 1), nude mouse splenocytes could incorporate only 592 ± 51 cpm of [^{3}H]thymidine; the background incorporation in the absence of concanavalin A was approximately 602 ± 181 cpm of [^{3}H]thymidine (Table 1). It thus seemed that infection of nude mice did not induce any recognizable

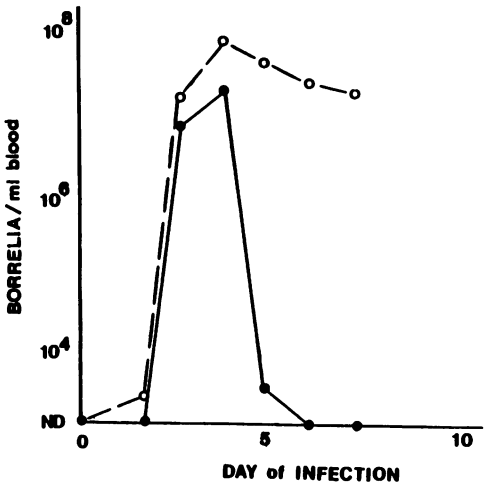

FIG. 2. Effect of cyclophosphamide on ability of the host to eliminate blood-borne *B. turicatae*. Symbols: ●, nontreated controls; ○, treated mice.

conversion of immature cells to functionally mature T lymphocytes.

Corticosteroids suppress the responsiveness of thymus-derived lymphocytes (15), and these drugs are routinely used to prolong survival of the pathogenic spirochete *Treponema pallidum* in experimental animals (20). When either dexamethasone or cortisone was employed to suppress elimination of borreliae, no effect upon the course of borrelemia was noted (Fig. 5). The peaks of infection and length of spirochetemias in treated mice compared favorably with those of untreated mice. Although no apparent effect upon elimination of spirochetes was observed with steroid treatment, weight loss was apparent in mice treated with dexamethasone, which resulted in a decrease of approximately 20% of the body weight of each animal; cortisone-treated animals lost less than 5% of their body weight during the course of each experiment. Untreated animals gained 40% additional weight during the experiment. The thymuses appeared to be atrophied in both sets of treated mice as compared with control animals (Table 2).

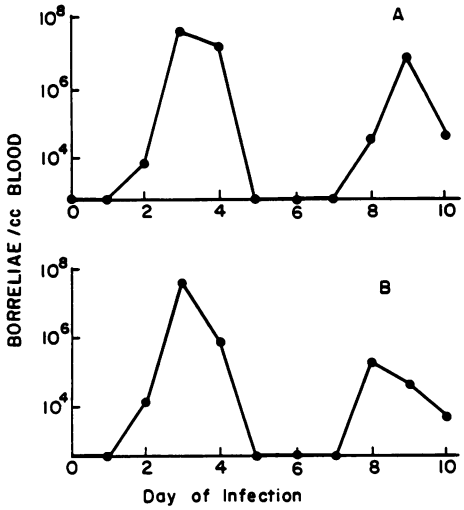

FIG. 3. Borrelemia in thymectomized outbred mice. (A) Sham littermates; (B) neonatally thymectomized animals.

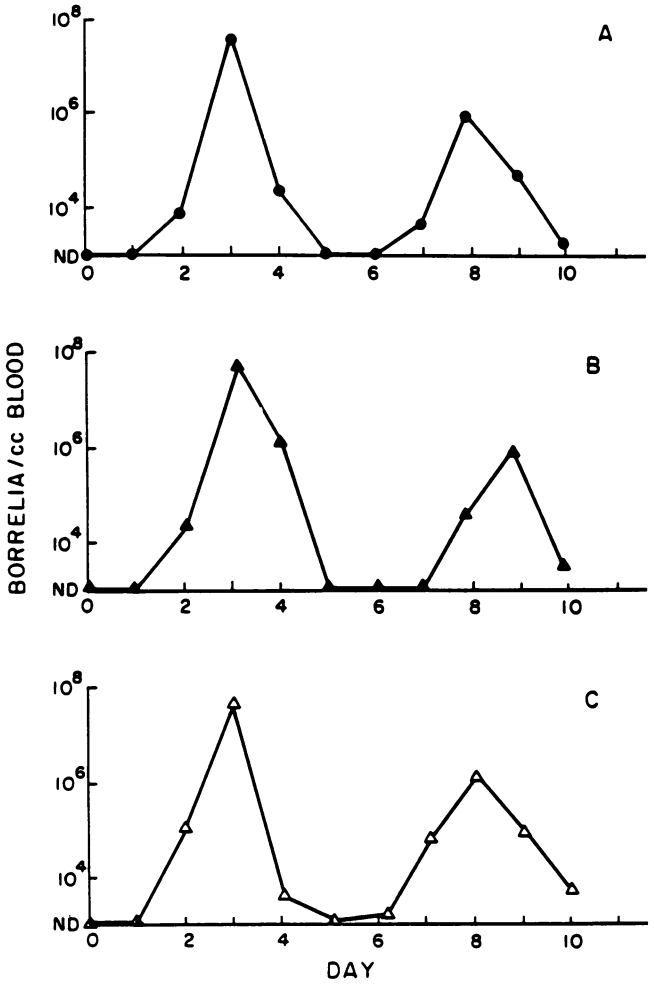

FIG. 4. Comparison of borrelemias in homozygous nude versus heterozygous or homozygous normal control mice. (A) BALB/c Wat one (*Nu/Nu*); (B) BALB/c one (*Nu/nu*); (C) BALB/c one (*nu/nu*).

To further define the lymphocyte requirements for an immune response, we used the technique of adoptive lymphocyte transfer. Nonimmune splenic lymphocytes were treated with anti-thy 1.1 or anti-immunoglobulin to selectively eliminate non-glass-adherent cells rich in T or B or both lymphocyte populations. Antibody production could occur in mice reconstituted with B lymphocytes (Table 3).

TABLE 1. Concanavalin A responsiveness of *Nu/nu* and *nu/nu* mouse lymphocytes isolated from infected animals[a]

Mouse strain	Status	Mitogen	Cpm per culture (mean ± SD)
Nu/nu	I	ConA	6,527 ± 612
(heterozygous normal)	U	ConA	7,402 ± 1,009
	U	LPS	3,012 ± 348
	U	—	602 ± 151
nu/nu	I	ConA	592 ± 51
(congenital athymic)	U	ConA	529 ± 235
	U	LPS	4,141 ± 511

[a] Cultures were 3 days old; labeling took place for 6 h. Abbreviations: I, infected; U, uninfected; ConA, concanavalin A; LPS, lipopolysaccharide (functional control). —, Background control.

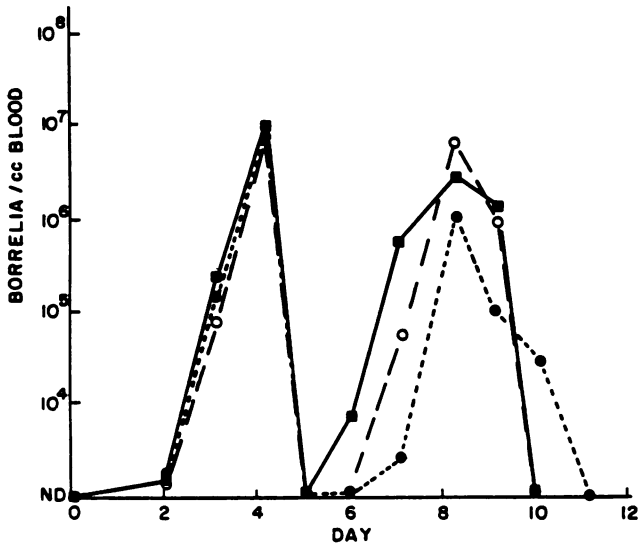

FIG. 5. Effect of steroid treatment on progress of borrelemias in outbred mice. Symbols: ●, controls; ○, cortisone-treated mice; ■ dexamethasone-treated mice.

DISCUSSION

Several previously reported observations have led us to speculate that the immune response to mammalian borreliae might be a T-cell-independent event. Cunningham (10) and Felsenfeld (12) have demonstrated that a given variant of borreliae could experimentally reinfect animals within a few months of recovery of the subject from infection. Similarly, Geigy (14) and others (5, 7) have noted that immunity to reinfection by a given local strain of *Borrelia duttoni* exists for only a short time after recovery.

Thymectomized and congenitally athymic mice have been noted for their lack of responsiveness to defined T-cell-dependent antigens (16, 19, 23). Merchant et al. (22) noted a 100-fold-lower responsiveness of nude mice to complex antigens such as bovine erythrocytes. Additionally, athymic mice have a demonstrated inability to eliminate many infectious agents, including *Listeria* and *Rickettsia* spp. (18). The study of Kishimoto et al. (18) showed that infection of nude mice by *Coxiella burnetti* elicited a T-cell-independent antibody response, although this response proved to be inadequate to promote elimination. Studies have also shown, however, that T-cell-deficient animals have the ability to mount a strong humoral response to a number of structurally simple polypeptides and polysaccharide (T-independent) antigens (1, 23). In light of our current understanding of the nature of the variant antigen of *B. turicatae*, the finding that T-cell-deficient mice could efficiently eliminate borreliae, whereas B-cell-deprived irradiated mice could not, strongly indicates that a T-cell-independent, B-cell-dependent immune mechanism is responsible for the elimination of *B. turicatae*.

TABLE 2. Effect of steroids on murine body weight and thymus size

Treatment	Mean body wt (g) on day:		% Change in body wt	Mean thymus wt (g) after treatment
	1	6		
None (control)	19.4	19.3	−1.3	0.235
Cortisone	18.8	17.1	−9.0	0.129
Dexamethasone	19.4	17.5	−9.8	0.052

TABLE 3. Antispirochetal antibody production in lymphocyte-reconstituted AKR/J mice

Lymphocyte	Detectable spirochetophilic serum components[a]		
	IgM	IgG	C3 Deposition
T[b]	−	−	+/−
B[c]	+	−	+
T + B[d]	+	−	+

[a] Direct fluorescent-antibody staining of blood-borne organisms. IgM and IgG, Immunoglobulins M and G, respectively; +, detected; −, not detected.
[b] B cells depleted with anti-immunoglobulin + C′.
[c] T cells depleted with anti-Thy 1.1 plus C′.
[d] Treated with C′ alone.

In seeming contradiction to the above conclusion is the report of DaMasso and Adler (11), in which it is noted that steroid treatment of young chickens prolonged the spirochetemia associated with *Borrelia anserina* infection. It must be pointed out, however, that single clones of *B. anserina* lack the multiphasic antigen repertoire observed in mammalian species (13). Thus, one might speculate that *B. anserina*, the type species for the genus *Borrelia*, might lack the variant antigen requisite for the mounting of a T-cell-independent response.

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