Chronological Development of Cellular Immunity in Human Toxoplasmosis

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A dichotomy between the resolution of clinical illness in acute toxoplasmosis and the responsiveness of B and T lymphocytes to toxoplasma antigens is described.

Infection of humans with the obligate intracellular protozoan *Toxoplasma gondii* evokes a cellular immune response which appears to play a major role in host defense against this parasite (5, 8). The present report describes a unique opportunity to study the cellular immune response in a patient both before and after naturally acquired toxoplasmosis. The subject had served as a normal noninfected control in previous studies of immunity in toxoplasma infection. He exhibited negative humoral and cellular responses to toxoplasma antigens during a 15-month period antedating his symptomatic infection. A serological response to toxoplasma antigen was documented within 1 month of his acute illness; however, his lymphocytes remained unresponsive to these same antigens in assays of blastogenesis and lymphokine production for 3 and 7 months, respectively.

Methods for the *in vitro* study of lymphocytes and monocytes were identical to those previously described (2). Briefly, mononuclear cells were isolated from Ficoll-Hypaque gradients, cultured in RPMI 1640 with 5% heated autologous serum and 20 μ g of protein per ml of soluble toxoplasma antigens for 3 days, and then pulsed with [³H]thymidine. Supernatants from these cultures were designated as lymphocyte products and incubated with adherent cells (monocytes) for 2 days before infection of the cells with living *T. gondii*. Infected monocytes were examined by phase-contrast microscopy 20 h after infection to determine the number of toxoplasmas per vacuole.

The clinical data and laboratory studies are shown in Table 1. This healthy 30-year-old male developed a 3-week self-limited illness characterized by fever, malaise, and posterior cervical lymphadenopathy in September. A diagnosis of toxoplasmosis was established by the documentation of a positive Sabin-Feldman dye test and a positive complement fixation test with titers of 1:16,000 and 1:8, respectively. The source of the

T. gondii organisms causing the infection was unknown. The patient's lymphocytes were not responsive to toxoplasma antigens in lymphocyte transformation tests until December, 3 months after he was completely asymptomatic. During this period, they did respond to the mitogens phytohemagglutinin, concanavalin A, and pokeweed mitogen. Inhibition of toxoplasma multiplication by monocytes after cultivation with lymphocyte products prepared from the patient's cells could not be demonstrated until 7 months after the clinical illness. The inability of the patient's monocytes to inhibit toxoplasma replication was not the result of a monocyte defect, since inhibition occurred (2.3 ± 0.1 toxoplasmas per vacuole) when the monocytes were preincubated with active lymphocyte products prepared from other subjects. Neither intracellular toxoplasma replication nor lymphocyte transformation tests were influenced by serum factors as determined in crossover studies utilizing heterologous sera with both positive and negative dye tests. The transformation of the patient's lymphocytes in response to toxoplasma antigen was also not enhanced by depletion of monocytes from the mononuclear cell preparation.

Delayed-type skin hypersensitivity (3), antigen-specific lymphocyte transformation (1, 6, 7), and lymphokine-induced monocyte inhibition and killing of ingested toxoplasmas (2, 11) have been documented in normal subjects after toxoplasmosis. In our previous study (4), the lymphocyte response to toxoplasma antigen of 16 subjects with negative Sabin-Feldman dye tests was 0.3 ± 0.1 cpm $\pm$ standard error of the mean $\times 10^{-3}$, whereas 18 subjects with positive dye tests for at least 1 year incorporated 2.6 ± 0.4 cpm. Similarly, the average number of toxoplasmas per vacuole 20 h after infection in monocytes preincubated with specific antigen-stimulated T-lymphocyte products was 2.4 ± 0.1 (1 standard deviation), whereas monocytes cultured with in-

TABLE 1. *Correlates of immunity in toxoplasmosis*

Mo	Serology (titer)		Lymphocyte transformation ^a (cpm of thymidine incorporated $\times 10^{-3}$)					Avg no. of toxoplasmas per vacuole in monocytes 20 h after infection ^b
	Sabin-Feldman dye test	Complement fixation test	Control	Phytohemagglutinin	Concavalin A	Pokeweed mitogen	Toxoplasma antigen	
June	Negative	Negative	0.1	5.2			0.1	3.5
September			0.1	35.8	19.6	7.5	0.4	4.0 ^c
February	Negative	Negative	0.1	16.7	9.2	18.1	0.1	3.8
September	— ^d	—	—	—	—	—	—	
October	1:16,000	1:8						3.3
November	1:16,000	1:8	0.2	20.6	13.9	4.7	1.3	
December			0.3	23.3	12.8	3.0	4.2	3.4
March	1:16,000	1:4						
April	1:4,096	Negative	0.3	37.4	12.6	4.0	6.6	2.4
May	1:1,024	Negative						1.9

^a Control lymphocytes were incubated with culture media alone.

^b Monocytes were cultured for 2 days with supernatants of autologous lymphocytes and toxoplasma antigen before infection with living *T. gondii*.

^c Preincubation of the patient's monocytes with lymphocyte products from another subject who responded to toxoplasma antigen in transformation tests resulted in 2.3 ± 0.1 toxoplasmas per vacuole.

^d —, Self-limited illness with fever, malaise, and cervical adenopathy.

active supernatants had 4.0 ± 0.3 toxoplasmas per vacuole (2). Thus, lymphocytes from healthy asymptomatic subjects with serological evidence of toxoplasmosis respond to toxoplasma antigens with increased incorporation of [³H]thymidine and the production of lymphokines which enhance monocyte function. Conversely, progressive or fulminant toxoplasmosis in immunosuppressed patients or fetuses has been attributed to their inability to mount an effective immune response (9).

The paradox in the present report is that the patient became asymptomatic months before cell-mediated immunity could be documented in vitro. Neither the development nor the resolution of the clinical illness was temporally related to his immune response. The eventual response of his lymphocytes to toxoplasma antigen and his excellent health during a subsequent 5-year follow-up period attest to his immunocompetence. The mechanism of this transient period of antigen-specific immunosuppression could not be attributed to serum or monocyte inhibitory factors. Recently, it has been shown that spleen cells from mice infected with *T. gondii* have a depressed response to a thymus-dependent antigen which may result from increased suppressor T-cell activity (E. A. Goidl, U. Ramstedt, and T. C. Jones, personal communication). This mechanism could explain the immune unresponsiveness in otherwise healthy patients after acute toxoplasmosis. The methodology is now available for the longitudinal study of lymphocyte subclasses in patients with toxoplasmosis (10).

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