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16. Contribution 334 from the Virginia Institute of Marine Science, Gloucester Point, Virginia.

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L-Asparaginase-Induced Immunosuppression: Effects on Antibody-Forming Cells and Serum Titers

Abstract. *Treatment of mice with* L-*asparaginase from* Escherichia coli *resulted in a marked suppression of the immune response, as assessed both cellularly and humorally. Suppression occurred only when the enzyme was injected together with the sheep erythrocytes used as antigen. There was little or no effect when the enzyme was injected before the antigen. Simultaneous injection of asparagine prevented suppression, an indication that the effect of the enzyme was due to depletion of an amino acid probably essential for normal lymphoid cell function during antibody production.*

L-Asparaginase, purified from *Escherichia coli,* can suppress the development of leukemia, lymphoma, or sarcoma in experimental animals (*1*). The efficacy of this enzyme in suppressing leukemic cells may reflect a unique difference between normal and tumor cells in the requirement for asparagine as an essential amino acid (*1, 2*). Maintenance of a relatively high concentration of the enzyme in the blood apparently prevents sufficient quantities of asparagine reaching the tumor cells, resulting in their "starvation." Thus, the enzyme does not seem to affect leukemic cells directly, but merely decreases the extracellular concentration of an essential nutrient (*1, 2*).

Most chemotherapeutic drugs used for treatment of leukemia and other neoplastic diseases also depress the immune response (*3*). Such agents are usually metabolic "poisons" or inhibitors directly affecting rapidly dividing cells. Although it is not known whether asparagine is an essential amino acid for normal leukocytes, it seemed plausible that administration of asparaginase to a normal animal could affect the response to an antigenic stimulus which stimulates rapid proliferation of specific immunocompetent lymphoid cells. Thus we attempted to determine whether injection of this enzyme into mice, at the time of antigen injection, would affect their immune response.

Mice were injected with relatively small doses of L-asparaginase (Worthington Biochemical Corp.) at a concentration known to affect leukemic cells (*1, 2*). These mice, as well as untreated controls, were then challenged with sheep erythrocytes, and the cellular and humoral immune responses were assessed. Individual antibody plaque-forming cells (PFC) appearing in the spleens of these mice were enumerated by the hemolytic immunoplaque assay in agar gel, essentially as described by Jerne *et al.* (*4*). Serum antibody was determined by microtitration (*4*).

Normal mice injected with sheep erythrocytes alone had a rapid appearance of specific PFC's in their spleens, with the peak number appearing on day 4 after immunization (Fig. 1). Mice treated with 10 international units (I.U.) of asparaginase before immunization had essentially the same response. However, when test animals were injected with the enzyme on the day of immunization and on the following 2 days, there was a diminution of the number of antibody-forming cells detected on day 4 and on subsequent days. In general, there was a 70 to 90 percent suppression of the peak number of PFC's in these animals, as compared to controls, either when calculated per whole spleen or per million spleen cells (Fig. 1 and Table 1). Injection of asparaginase during the first 4 days after immunization resulted in almost a complete suppression of the PFC response (Fig. 1).

The effect of enzyme dose and time of injection was also studied. Mice receiving one injection of enzyme either the same day or 1 or 2 days before immunization had only a slight to moderate decrease in the number of PFC's detected 4 days later (Table 1). Two injections on days 0 and 1 resulted in a significant suppression. However, the greatest suppression occurred in mice treated with the enzyme during the first 3 or 4 days after immunization. In addition, a greater degree of suppression occurred with 10 or 50 units of enzyme, as compared to 0.5 or 5 units.

Serum antibodies were most suppressed in mice treated with enzyme on the day of immunization and the following 2 to 4 days (Table 1). One injection of enzyme had a slight to moderate effect on the titers, which were generally parallel to the effects

Table 1. Effect of time and dose of asparaginase administration on antibody response to sheep erythrocytes and spleen weight 4 days after challenge immunization. The PFC response is the average response of five or more mice per group; the differences between animals within a group was never greater than ± 30 percent.

Time	Asparaginase per injection (I.U.)	PFC response		Spleen weight (percent of control)	Serum titer (mean)
		Per spleen	Per 10^6 spleen cells		
		None			
		57,700	237	100	1:220
		Single injection of asparaginase			
Day −2	10	38,640	207	76	1:106
Day −1	10	26,290	155	66	1:64
Day 0	10	22,720	148	71	1:16
Day 0	50	10,950	59	68	1:6
		Multiple injections of asparaginase			
Days 0, +1	10	7,490	47	61	<1:2
Days 0, +1, +2	10	5,000	24	55	<1:2
Days 0, +1, +2, +3	10	2,850	3	50	<1:2
Days −6, −5, −4	10	54,000	231	100	1:200
Multiple injections of asparaginase plus 10 mg of asparagine per day					
Days 0, +1, +2, +3	10	50,650	206	92	1:235
		*Inactivated asparaginase**			
Days 0, +1, +2, +3	10	53,300	238	95	1:158

* Enzyme solution agitated in glass flask or heated at 80°C for 30 minutes; 90 to 95 percent or more of the activity was decreased.

observed with the single cell assays (Table 1).

Inactivation of enzyme activity by heat or surface denaturization abolished its effect (Table 1). Furthermore, simultaneous administration of 10 mg of asparagine daily to each animal, along with the enzyme, generally reversed the inhibitory effect of the enzyme.

Treatment of mice with L-asparaginase at the time of immunization may interfere with formation of antibody by individual cells. It seems unlikely that the enzyme affects the immune response merely by destruction of leukocytes or their precursors. A significant decrease in spleen size and cell number occurred only after multiple injections of the enzyme. Cessation resulted in a rapid restoration of the spleen weight.

The effect of enzyme treatment on antibody-forming cells could be due to "starvation" of rapidly dividing cells involved in the immune response, a mechanism similar to that proposed for the suppression of leukemic cells by asparaginase (*2*). Maintenance of relatively high concentrations of this enzyme in the blood reduces the amount of circulating asparagine (*1*, *2*). Lymphocytes and plasma cells involved in the immune response would thus be unable to obtain a sufficient quantity of this specific amino acid and would be unable to participate in the complex sequence of cellular and chemical events involved in antibody synthesis. Reversal of the inhibitory effect of asparaginase by large doses of asparagine is compatible with this hypothesis. The possibility also exists that the immunosuppressive effect of this enzyme from *E. coli* is due to nonspecific "antigenic competition" or to toxic properties of bacterial endotoxins, which undoubtedly contaminate the preparation. However, it would be expected that the two last-mentioned mechanisms would result in immunosuppression only when enzyme was injected before and not after injection of red blood cells (*5*).

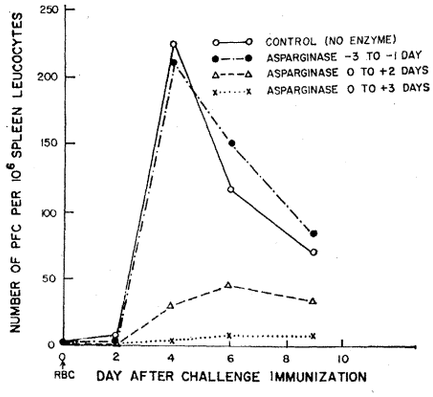

Fig. 1. The cytokinetics of the antibody plaque response to sheep erythrocytes in spleens of control mice and mice treated with *E. coli* L-asparaginase (10 I.U.) either before or after injection of red blood cells. Each point represents the average number of PFC's per 10^6 spleen cells of five or more mice. All animals were immunized intraperitoneally with 4×10^8 red blood cells (RBC) on day 0.

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Tolerance to Polyinosinic • Polycytidylic Acid in NZB/NZW Mice

Abstract. *Immunological tolerance to polyinosinic • polycytidylic acid was induced in NZB/NZW mice. This is the first experimental induction of tolerance to a nucleic acid antigen.*

The disease of NZB/NZW F_1 (B/W) mice is a useful model for human systemic lupus erythematosus. Both mice and humans develop antibodies to DNA, deposit DNA-containing immune complexes in their kidneys, and die of glomerulonephritis. The murine disorder is strongly influenced by genetic factors (*1*). These factors may operate through altered immunologic regulation leading to augmented antibody responses and inability to develop or maintain immunologic tolerance (*2*). These mice carry murine leukemia virus and make antibodies to viral antigen (*3*).

Treatment of B/W mice with multiple injections of fluid polyinosinic • polycytidylic acid (poly I • poly C) caused production of interferon, but the mice became immunized to the double-stranded RNA and had an accelerated onset of antibodies to DNA and of nephritis (*4*). Control mice treated with fluid poly I • poly C did not make antibodies to RNA or DNA.

Because antibodies to nucleic acids are important in the pathogenesis of murine and human systemic lupus erythematosus (*5*), we considered the induction of specific immunological tolerance or suppression to prevent their formation. Both corticosteroids and cyclophosphamide are efficacious in the therapy of New Zealand mice (*6*). The therapeutic approach of specific tolerance in systemic lupus erythematosus might avoid many of the problems of toxicity and infection that occur with standard current therapy, long-term corticosteroids, or immunosuppressive agents. The finding that poly I • poly C acts as a specific in-

Table 1. Tolerance to poly I • poly C in 4-week-old female B/W mice given cyclophosphamide (60 μg/g) 24 hours after receiving fluid poly I • poly C (100 μg) and challenged with poly I • poly C in complete Freund's adjuvant. The untreated controls were not challenged with poly I • poly C in complete Freund's adjuvant.

Treatment	Mice (No.)	Poly I • poly C binding capacity (μg/ml)
Poly I • poly C + cyclophosphamide		
3 courses	8	< 0.1
2 courses	8	< 0.1
1 course	8	1.1
Cyclophosphamide alone	12	11.0
Poly I • poly C	12	12.4
Controls	6	11.5
Untreated controls	16	0.1