Reversal of Antilymphocytic Serum-Induced Immunosuppression By Macrophage Administration[1] (35153)

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The key role of the macrophage as the afferent component of the immune reflex arc is well documented (6–8, 10, 11, 19, 22, 31). Furthermore, Frei *et al.* (9) demonstrated that phagocytosis is an essential step in the development of the primary immune response while others have shown that the transplantation of macrophages into newborn mice (1) and rabbits (19) will promote the early development of immunocompetence. In view of these findings, it would be expected that the administration of chemotherapeutic agents which alter the normal phagocytic function of the reticuloendothelial system (RES) would induce a corresponding alteration in the immune response. Indeed, Wooles and Di Luzio (31) initially demonstrated that RES depression was associated with a decreased immune response while RES stimulation resulted in an increased antibody titer.

Previous studies from this laboratory (25) have shown that a single injection of antilymphocytic serum (ALS) impairs phagocytic activity of the reticuloendothelial system (RES), as reflected by a depressed intravascular clearance rate of gelatinized RE test lipid emulsion. The ALS-induced depression in phagocytosis was associated with a selective decrease in Kupffer cell phagocytosis. *In vitro* studies which employed both serum and liver slices derived from either normal or ALS-treated animals also indicated the ability of ALS to induce phagocytic impairment. These *in vitro* studies (25) suggested that the depression of RES activity by ALS could be attributed to an exclusive defect in the cellular compartment of the RES since no alteration in circulating phagocytic-promoting activity was found in ALS-treated animals. Furthermore, a direct injurious effect of ALS on Kupffer cells was demonstrated, not only by ALS-induced impairment of Kupffer cell phagocytic activity, but also by the cytotoxic action of ALS on these macrophages (25). These studies suggested the distinct possibility that ALS-induced immunosuppression may be mediated by an alteration of RES function which results from the antimacrophage activity manifested by ALS (25).

Antilymphocytic serum has been shown to be an effective immunosuppressant of both cellular (13, 20, 29) and humoral immune responses (14, 20, 29). Indeed, ALS has been employed extensively in renal transplantation (23, 28, 31) despite the fact that the mode of action of ALS-induced immunosuppression is as yet unclarified (13, 15, 16, 20, 26). In view of our previous observations of the detrimental effect of ALS on Kupffer cell viability and function (25), studies were undertaken to evaluate the influence of hepatic as well as peritoneal and splenic macrophage transplantation on ALS-induced immunosuppression. Studies were also extended to determine the ability of splenic and thymic lymphocytes to restore the humoral antibody response in ALS-immunosuppressed animals.

Materials and Methods. Horse anti-rat lymphocyte serum (ALS) and normal horse serum (NHS) were obtained from Microbiological Associates, Inc. (Bethesda) in lyophilized form. The ALS was reconstituted and stored at —20° prior to use. In all experiments Sprague-Dawley rats weighing between 250 and 300 g were employed.

Rats received five daily intravenous injections of either 1 ml of ALS or NHS. On day 5, the rats received, intraperitoneally, 2 ×

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10^8 sheep erythrocytes (SRBC). Control rats received 1 ml of saline while experimental animals received either 2×10^7 macrophages or 2×10^7 lymphocytes injected intraperitoneally. The rats were bled 7 days after antigenic challenge. Serum was collected, heat-inactivated for 30 min at 56°, and titered for hemolysin activity. Serial 2-fold dilutions of the test serum were done using modified barbital buffer (pH 7.4) as diluent. Lyophilized guinea pig serum, reconstituted with 6% sodium acetate in 2% aqueous boric acid solution, was used as a complement source. The tubes were incubated for 30 min at 37°, then centrifuged. Optical densities were determined, and the 50% hemolysin titer calculated.

Rat macrophages were obtained from normal, untreated rats. Peritoneal macrophages were collected 4 days after an intraperitoneal injection of 10 ml of a 12% sodium caseinate solution. Splenic macrophages were harvested by the adsorption method described by Mosier (21). Kupffer cells were isolated by the method of Pisano *et al.* (24). The peritoneal and splenic macrophage populations had an average of 20% and 30% lymphocyte contamination, respectively. Histological examination of the isolated Kupffer cells demonstrated a 30–40% contamination with lymphocyte-like cells.

Lymphocytes were isolated from dispersed splenic cells by allowing the macrophages to adsorb onto petri plates as described by Mosier (21). Thymocytes were isolated by forcing minced thymus tissue through a 10xx silk screen. Qualitative histological examination of the lymphocyte cellular preparations revealed that a slight (<10%) macrophage contamination occurred.

Results. The intraperitoneal injection of SRBC into untreated control rats resulted in an average hemolysin titer of 1:67 (Table I). The administration of NHS prior to antigenic challenge was associated with a 37% reduction in antibody titer. In contrast to the results obtained with NHS-treated animals, intravenously injected ALS produced a 90% reduction in the antibody titer of recipient animals to SRBC (Table I).

Transplantation of either thymocytes, splenic macrophages or lymphocytes, peritoneal macrophages, or Kupffer cells did not alter the hemolysin titer produced by the injection of SRBC into untreated control rats. Similarly, the transplantation of these isolated cell populations did not induce a hemolysin response in animals which did not receive SRBC.

TABLE I. Effect of Antilymphocytic Serum on Rat Anti-SRBC Antibody Production.

Treatment	50% Hemolysin titer	
	Individual	Average
None	1:33	1:67
	1:91	
	1:55	
	1:69	
	1:91	
	1:63	
ALS[a]	1:9	1:6
	1:6	
	1:5	
	1:5	
	1:3	
	1:7	
NHS[b]	1:29	1:42
	1:63	
	1:32	
	1:38	
	1:33	
	1:59	

[a] Animals received 1 ml ALS (iv) daily for 5 days prior to injection of SRBC.

[b] Animals received 1 ml NHS (iv) daily for 5 days prior to injection of SRBC.

Cellular reconstitution studies employing the passive transfer of thymocytes and splenic lymphocytes into ALS-treated rats indicated that neither thymocytes nor splenic lymphocytes were capable of reversing the immunosuppressive action of ALS (Table II). The 82% and 77% reduction in hemolysin titer in ALS-treated rats that received SRBC and either thymocytes or splenic lymphocytes, respectively, was not significantly different from the 90% reduction in the immune response observed in rats which received ALS alone.

In contrast to the inability of thymocytes and lymphocytes to restore immune responses of ALS-treated rats, the transplantation of

TABLE II. Lack of an Effect of Lymphocyte Transplants on ALS-Induced Humoral Antibody Suppression.

Treatment	50% Hemolysin titer	
	Individual	Average
Saline[a]	1:174	1:89
	1:32	
	1:76	
	1:73	
ALS[a]	1:9	1:9
	1:2	
	1:9	
	1:17	
ALS + splenic[b] lymphocytes	1:26	1:21
	1:44	
	1:10	
	1:2	
	1:20	
ALS + thymic[b] lymphocytes	1:8	1:11
	1:7	
	1:6	
	1:17	
	1:17	

[a] Animals received 1 ml saline or ALS (iv) daily for 5 days prior to receiving SRBC.

[b] Animals received 2×10^7 lymphocytes or thymocytes plus SRBC after 5 daily injections of ALS.

the various macrophage populations into ALS-treated animals resulted in either a complete or partial reversal of ALS-induced immunosuppression (Table III). Peritoneal macrophages were capable of completely reversing the immunosuppressive effects of ALS on hemolysin formation. Both splenic macrophages and hepatic macrophages were capable of partially reversing the immunosuppressive activity of ALS. In this regard, splenic macrophages were capable of inducing a 67% reversal in ALS immunosuppression while the Kupffer cell transplantation was associated with a 58% restoration of immune activity.

Discussion. Most hypotheses employed to explain ALS-induced immunosuppression are based upon the antilymphocytic activity of the antiserum (6, 16, 20, 29). Recent studies have demonstrated that ALS possesses anti-Kupffer cell (25) and antiperitoneal macrophage activity (5, 12, 17, 18). The antimacrophage activity of ALS has been demonstrated *in vivo* with intravascular clearance techniques (12, 17, 25) and *in vitro* with either liver slices (25) or isolated phagocytic cell populations (5, 18, 25). Thus, it is highly possible that part of immunosuppressive activity of ALS, as X-irradiation (4, 10) and cortisone (2), is the result of a depression in macrophage function.

The concept that ALS-induced immunosuppression is mediated by the macrophage was supported by the demonstration that the administration of hepatic, peritoneal, or splenic macrophages was capable of either

TABLE III. Reversal of ALS-Induced Suppression of Rat Anti-SRBC Antibody Production by Macrophage Reconstitution.

Treatment	50% Hemolysin titer	
	Individual	Average
Saline	1:67	1:66
	1:72	
	1:65	
	1:63	
	1:65	
ALS[a]	1:9	1:7
	1:9	
	1:6	
	1:4	
	1:8	
ALS + peritoneal macrophages[b]	1:56	1:65
	1:89	
	1:91	
	1:31	
	1:48	
	1:74	
ALS + splenic macrophages[b]	1:73	1:43
	1:73	
	1:24	
	1:10	
	1:50	
	1:25	
ALS + Kupffer cells[b]	1:26	1:37
	1:6	
	1:34	
	1:22	
	1:96	

[a] Animals received 1 ml saline or ALS (iv) daily for 5 days prior to receiving SRBC.

[b] Animals received 2×10^7 of appropriate macrophages plus SRBC after 5 daily injections of ALS.

partially or completely restoring the immune response in ALS-immunosuppressed rats. Since all three macrophage populations consisted of at least 20% lymphocyte-like cells, the lymphocyte cannot be conclusively excluded as the cellular target responsible for ALS immunosuppression. However, since populations of cells rich in lymphocytes, namely, the splenic and thymic lymphocytes, did not promote immunogenesis in ALS-treated animals, it would appear that the primary ALS-induced immunosuppressive defect is not mediated by the lymphocyte. Indeed, these results would clearly suggest that ALS immunosuppression is mediated by the macrophage.

Fishman (6, 7) has shown that the macrophage has a definitive role in the initiation of immunogenesis through its antigen-processing activity, a concept which has since been confirmed and extended by many investigators (8, 11, 19, 22, 31). Recently, Frei *et al.* (9) has presented evidence which indicates that immunogenesis after administration of particulate antigens cannot occur in the absence of phagocytosis. In view of the demonstrated role of the macrophage in immune phenomena, the cytotoxic effect of ALS on macrophages (5, 12, 17, 18, 25) would be expected to alter not only the phagocytic event but also modify antigenic processing.

The present hypothesis that ALS immunosuppression is mediated, in part, by the macrophage is not unique to the mode of action of immunosuppressive agents. Feldman and Gallily (4) have demonstrated a similar mechanism for X-irradiation-induced immunosuppression. Both cycloleucine (10) and cyclophosphamide (27) have been shown to possess antimacrophage activity and it has been suggested that the mode of immunosuppressive activity of these agents may also be mediated by the RES. Similarly, methyl palmitate has been demonstrated to induce a significant immunosuppression by altering RES function as determined by intravascular clearance techniques of particulate antigens (31). Furthermore, cortisone-induced reduction in the number of splenic antibody-producing cells can be restored to normal levels after macrophage transplantation.

In view of the fact that patients receiving immunosuppressive therapy are more susceptible to infection (30) and to neoplastic growth (23), it is possible that the anti-RES activity of such therapeutic drugs as ALS may be a mechanism in increased susceptibility to infectious agents and neoplastic growth. Depression of the RES in mice receiving homologous tumor cell transplants is associated with impaired rejection mechanisms (32), thus the maintenance of normal macrophage activity after immunosuppression may be an important factor in preventing infection and neoplasia.

The present observations demonstrated that the transplantation of lymphocytes and thymocytes was incapable of reversing ALS-induced immunosuppression, while the administration of hepatic, splenic, or peritoneal macrophages either partially or completely reversed ALS-induced suppression of the immune response. These findings extend our previously proposed concept that the macrophage is a primary cellular site of ALS-induced immunosuppression.

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