data, rests unconformably upon volcanic rocks of unknown age; it was eroded to provide detritus for early Late Eocene conglomerates.

Other areas of Early and Middle Eocene volcanic activity in the Caribbean are a center including easternmost Cuba (*12*), eastern Jamaica (*13*), and northwestern Haiti (*14*), and another center in the northeastern Caribbean near St. Bartholomew (*15*). A lower Tertiary volcanic center near Tobago has been suggested (*14*), on the basis of the correlation of undated dacite and andesite volcanic rocks deformed in a pre-Miocene orogeny (*16*). As dacitic volcanism occurs elsewhere in the Caribbean in the latest Cretaceous and early Tertiary, this correlation may be tentatively accepted. The generally dacitic and andesitic volcanism of this age is more silicic than the Middle and Upper Cretaceous andesitic and basaltic volcanism, and also more silicic than the post-Eocene basalt-andesite volcanism of the Lesser Antilles. Volcanic activity occurred elsewhere around the North Atlantic Ocean during early Tertiary time—for example, in Scotland, Ireland, and Greenland. This activity was dominantly basaltic, however, and probably not directly related to layer A.

Thus, a Caribbean source for siliceous material is provided in Early and Middle Eocene times by a series of volcanic centers near eastern Cuba, near the northern Dominican Republic, in central Puerto Rico, near St. Bartholomew, and possibly near Tobago. Pyroclastic deposits or components of deposits from these eruptive centers occur in western Cuba and along the eastern coast of North America. Layer A in the North Atlantic and layer A″ in the Caribbean (*17*) probably represent the deep-water equivalent of these pyroclastic deposits—radiolarian-bearing cherty rocks. Distribution of the volcanogenic material to the north and east of the volcanoes could be due in part to atmospheric dispersal and in part to movement by ocean currents such as the Gulf Stream, as suggested by several authors (*3, 4*).

Thick Eocene deposits, coarse-grained volcanic fragments, and lavas have not yet been found in layer A or A″. They seem to be restricted to the island exposures, owing in part to the natural increase in coarseness and volume near volcanic vents. In addition, the apparent sharp landward boundaries of layers A and A″, shown in profiles crossing the Greater Antilles (*4*), may have been produced when ancient trench systems trapped the bulk of the volcanic deposits, thus permitting only fine current- or air-borne ash to enter the main ocean basins.

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17. Results from legs 4 and 5 of the JOIDES program demonstrate that layer A in the western North Atlantic is lithologically and chronologically equivalent to layer A″ in the Caribbean. The Maestrichtian age originally determined by Saito and others [see (*1*)] must have been from strata below layer A. A complete discussion of these correlations is given by J. Ewing, C. Windisch, and M. Ewing [*J. Geophys. Res.* **75**, 5645 (1970)].
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L-Asparaginase Induced Immunosuppression: Inhibition of Bone Marrow Derived Antibody Precursor Cells

Abstract. *The cooperation between bone marrow and thymus cells in restoring the hemolytic antibody response to sheep erythrocytes in immunosuppressed recipients was markedly inhibited when donor mice were treated with* L-*asparaginase, a known inhibitor of lymphocyte function. The marrow cell population was shown to be a major target for the immunosuppressive activity of asparaginase, since thymus cells from enzyme-treated animals interacted with marrow cells from normal animals to generate immunocompetent cells.*

L-Asparaginase (L-asparagine amidohydrolase) is a potent inhibitor of many malignant lymphoreticular cell tumors, both in vitro and in vivo (*1*). Furthermore, lymphoid cells incubated in vitro with this enzyme lose most of their ability to undergo blastogenic transformation when stimulated with phytohemagglutinin or antigens (*2*). Other recent studies have shown that treatment of mice, rats, or rabbits with asparaginase markedly depresses the immune response to sheep erythrocytes, *Escherichia coli* somatic antigen, serum proteins, or skin allografts (*3*). However, the mechanism behind the immunosuppressive effects of this enzyme is still unkown. A plausible working hypothesis suggests that relatively high levels of exogenous L-asparaginase in the circulation of an individual prevents sufficient quantities of asparagine from reaching rapidly dividing lymphoid cells, either malignant or those stimulated by antigen, thus resulting in their "starvation" (*1–3*). Nevertheless, the exact mode of action of this enzyme as a suppressor of either neoplastic cells or immunocytes is not known.

Adoptive cell transfer procedures with lymphoid cells are often used in experimental models designed to determine cell sources involved in specific immune responses. Recent studies from a number of laboratories have produced ample evidence that several cell types may "cooperate" in the immune response, especially when thymus-dependent antigens are involved (*4*). For example, it is now quite clear that cell populations derived from the thymus and bone marrow may interact in vivo to generate immunocompetent cells to sheep erythrocytes when transferred to

Table 1. Immunosuppressive effect of L-asparaginase; inhibition of antibody PFC response in prospective donor mice given graded doses of enzyme after immunization with sheep erythrocytes.

Mouse group*	Number of antibody PFC's per spleen on day†						Mean peaks serum titer (log_2)
	0	2	4	7	10	15	
Normal (saline controls)	<100	1,750	36,800	12,640	3,500	1,820	1 : 286
L-Asparaginase treated							
50 I.U. daily	<100	630	1,120	835	265	200	<1 : 4
20 I.U. daily	<100	965	2,100	1,730	2,150	475	1 : 4
10 I.U. daily	<100	1,130	4,800	1,650	980	865	1 : 16
5 I.U. daily	<100	1,850	21,500	6,500	5,830	2,150	1 : 96
1 I.U. daily	<100	1,630	28,500	14,100	3,950	2,130	1 : 198

* Mice injected intraperitoneally with indicated dose of enzyme during each of first 4 days after intraperitoneal immunization with 4×10^8 sheep erythrocytes. † Average response of three to five mice per group on day indicated after immunization.

x-irradiated recipients. An adaptation of this model was utilized in the present experiments to study cell types involved in the immunosuppressive phenomenon observed when L-asparaginase is given to mice challenged with sheep erythrocytes as the test antigen.

For these experiments adult Balb/c mice weighing approximately 20 g were given intraperitoneal injections of L-asparaginase, generally at a dose of 20 to 40 international units (I.U.) per animal. Lymphoid cells derived from the spleen, lymph nodes, thymus, and bone marrow of enzyme-treated mice, as well as such cells from the same tissues of control, nontreated mice, were transferred intravenously to syngeneic recipient mice treated 24 hours earlier with 850 r of whole body irradiation (*5*). The mice were then challenged by intraperitoneal inoculation of 4×10^8 sheep erythrocytes. The animals were killed 8 to 9 days later and the number of antibody plaque-forming cells (PFC's) in the spleens of these recipients was determined by the direct hemolytic plaque assay in agar gel exactly as described elsewhere (*6*). The number of zones of hemolysis on three or more plates for each spleen cell suspension was enumerated and used to calculate the average number of PFC's per recipient group.

As can be seen from Table 1, treatment of donor mice with L-asparaginase during the first 2 to 4 days after immunization resulted in a 90 to 95 percent or greater depression of the expected antibody plaque response. This suppression was dose dependent in that the maximum effect occurred in mice given the largest dose of enzyme. Spleen cells from mice treated with asparaginase were transferred to irradiated recipients, which were then challenged with the erythrocytes. These recipients showed a marked deficiency in appearance of PFC's, as compared to other recipients given spleen cells from normal, nontreated donors (Table 2). Similarly, lymph node cells from L-asparaginase–treated donors were also markedly deficient in transferring antibody-producing capacity to recipient mice.

Thymus or bone marrow cells alone, from either normal or enzyme-treated donors, were incapable of transferring antibody responsiveness to recipients. However, as can be seen from Table 2, transfer of equivalent numbers of *both* bone marrow and thymus cells from normal donors to irradiated recipients resulted in a marked appearance of PFC's; transfer of similar numbers of these cell types from L-asparaginase–treated donors resulted in essentially no PFC's. Transfer of thymus cells from L-asparaginase–treated donor mice with an equal number of bone marrow cells from normal animals induced a rapid and typical appearance of PFC's in the recipients, similar to the results obtained with joint transfer of normal thymus plus normal marrow cells (Table 2). In contrast, bone marrow cells from enzyme-treated donors were not capable of interacting with thymus cells from normal donors to restore immunocompetence in recipient mice. Very few PFC's appeared in the spleen of such recipients.

The results of these experiments indicate that the "target" cells for the immunosuppressive property of asparaginase are the antibody precursors present among the bone marrow cell population. Bone marrow and thymus cells from normal donors "cooperate" in restoring immunocompetence in animals suppressed by x-irradiation prior to challenge immunization with sheep erythrocytes, an antigen considered to be "thymus dependent." Transfer of both bone marrow and thymus cells from normal donors into irradiated recipients restores the PFC response to essentially the same level as that which occurs following transfer of cells from the spleen, an organ considered to contain cells derived from *both* the bone marrow and thymus. In the present experiments thymus cells from donor mice immunosuppressed with asparaginase "cooperated" with bone marrow cells from normal donor mice and restored PFC formation. Since similar recipients given thymus cells from normal donors plus bone marrow cells from enzyme-treated mice could not restore immune competence in irradiated mice, it seems plausible that the bone marrow cell population was the major target of the L-asparaginase in regard to immunosuppression.

Table 2. Effect of transfer of spleen, thymus, and bone marrow cells from normal or L-asparaginase-treated donor mice on antibody plaque response of x-irradiated recipients immunized with sheep erythrocytes.

Cells transferred*	Recipient PFC response on day†		
	6	9	12
None	<100	<100	<100
Normal mice			
Spleen	1120	2950	975
Thymus	<100	<100	<100
Bone marrow	<100	198	118
Thymus + bone marrow‡	135	2700	1950
L-Asparaginase–treated mice			
Spleen	<100	240	110
Thymus	<100	<100	<100
Bone marrow	<100	<100	<100
Thymus + bone marrow‡	<100	<100	<100
Normal bone marrow + L-asparaginase–treated thymus‡	980	2180	1180
L-Asparaginase–treated bone marrow + normal thymus‡	180	275	260

* Approximately 50×10^6 nucleated lymphoid cells from indicated tissue of donor mice, either normal or treated daily for 4 days with 20 I.U. of L-asparaginase, transferred intravenously into groups of 10 to 20 recipient mice treated 24 hours earlier with 850 r of whole body irradiation; all mice were challenged immediately after cell transfer with 4×10^8 sheep erythrocytes. † Average PFC response in spleens of three to six mice per group on day indicated after cell transfer and immunization. ‡ Total of 50×10^6 cells transferred (25×10^6 of each cell type).

Further studies with this model system, including transfer of cells into enzyme-treated mice, should permit a more rational analysis of the mode of action of this enzyme as it affects various parameters of the immune mechanism, including humoral and cellular responses.

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Kidney: Primary Source of Plasminogen after Acute Depletion in the Cat

Abstract. *The kidney was the primary source of plasminogen to restore normal plasma levels, after acute plasminogen depletion was produced by injection of streptokinase in cats. The concentration of plasminogen in the hepatic vein remained below that in the artery during the time when concentrations in the artery and renal vein were returning to normal.*

Plasminogen (profibrinolysin), a plasma protein, is the precursor of the proteolytic enzyme, plasmin (fibrinolysin). The primary sites of synthesis or storage (or both) of plasminogen have not been firmly established in any species. Perfusion of isolated rat livers (*1*) yielded negative results, and use of the fluorescent antibody technique by Barnhart and Riddle (*2*) showed that plasminogen was localized in the eosinophilic granules of bone marrow cells. The extremely small number of eosinophils in the circulating blood, compared to the relatively high concentration of plasminogen in plasma, led us to look elsewhere for possible sites of plasminogen production.

Adult male cats were anesthetized with 5 percent pentobarbital sodium (0.7 ml per kilogram of body weight, intraperitoneally), and a control sample of arterial blood was obtained by left cardiac puncture. All cats were then rapidly infused through the left femoral vein with 10^6 units of streptokinase (SK) (*3*) in 3 ml of sterile saline. Streptokinase is an effective activator of plasminogen in this species, as well as in humans and other primates (*4*). Arterial and venous samples were taken within a few minutes after infusion in order to verify the depletion of circulating plasminogen. Samples were taken from some animals hourly; and the animals were given supplementary pentobarbital as needed. Some animals were permitted to recover from the anesthetic and were reanesthetized for later sampling. Arterial blood was obtained by cannulation of the left femoral artery, and venous blood was obtained by venipuncture. All samples were treated and assayed as follows. The euglobulin fraction was precipitated by 20-fold dilution of the plasma with distilled water and acidification to *p*H 5.3 with 2 percent acetic acid. The resulting precipitate was collected by centrifugation, and was suspended in 0.05*M* H_2SO_4 in a volume equal to that of the original plasma sample (*5*). After extraction for 10 minutes, the suspension was centrifuged, and the supernatant was analyzed for plasminogen and plasmin by α-caseinolytic assay at *p*H 7.4 according to the specifications of the Committee on Thrombolytic Agents (CTA) (*6*). Plasminogen activity was calculated by subtracting spontaneous plasmin activity from the total activity measured after addition of SK to the assay. Completeness of activation in the assay was verified by the use of urokinase in parallel with SK in duplicate samples obtained before and after SK infusion. To insure that plasminogen was completely precipitated in the euglobulin fraction and that true changes in plasminogen content were being measured, we used an alternate method of treating samples without precipitation of plasminogen. Acidification and neutralization of whole plasma (*7*) obtained before and after SK infusion gave the same values for circulating plasminogen as those found with the euglobulin precipitation method. Results are expressed in CTA units per milliliter of plasma.

After the infusion of SK, plasminogen in arterial blood dropped within 30 minutes from 5 CTA units per milliliter of plasma to unmeasurable levels.

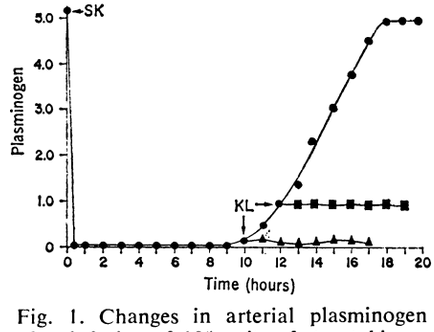

Fig. 1. Changes in arterial plasminogen after infusion of 10^6 units of streptokinase (SK). Results are expressed as CTA units per milliliter of plasma. Solid dots (●) represent the means in at least three animals. Squares (■) indicate levels in one animal after kidney vessel ligation (KL) at 12 hours. Triangles (▲) indicate levels in one animal after kidney ligation at 10 hours. Times of infusion and ligation are indicated by arrows.

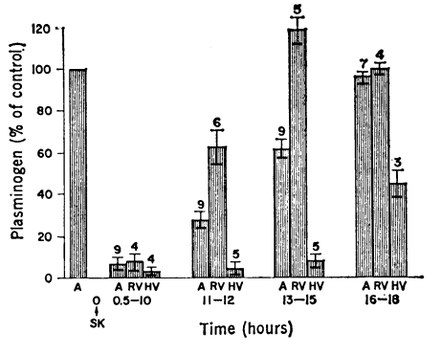

Fig. 2. Changes in arterial (A), right renal vein (RV), and hepatic vein (HV) plasminogen concentrations at different time intervals after infusion of streptokinase (SK). Results are expressed as percentage of control arterial values with mean ± standard error indicated by vertical bars. Number of animals sampled at each time period is shown above the vertical bars.