Suppression of Established Friend Virus Leukemia by Statolon V. Reversal of Virus-Induced Immunodepression to Sheep Erythrocytes[1] (37032)

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Statolon, an extract of the mold *Penicillium stoloniferum* containing a double stranded RNA mycophage, can convert a rapidly lethal Friend leukemia viral (FV) infection into a dormant one (1–5). One inoculation of statolon, administered when FV is disseminated throughout the body, induces interferon and FV-cytotoxic antibody thereby suppressing both virus and virus-transformed cells (2, 5). This suppression results in a long lasting clinical remission during which no infective Friend virions can be detected (1, 4). The FV genome is present however, as shown by the spontaneous emergence of FV leukemia late in the life of mice with dormant infections and by the leukemogenicity of spleen cells transferred from mice with dormant infections to normal mice (3).

Interferon production leads to suppression of FV replication, but by itself cannot completely suppress the disease; poly I:C and Newcastle disease virus can produce as much interferon as statolon, clear the blood of FV but cannot convert FV leukemia into a dormant infection (2).

Production of humoral antibody against both virus and virus transformed cells appears to be crucial for development of the FV-dormant state. Untreated FV leukemic mice have no demonstrable antibody and are considered immunosuppressed in this regard, although high titers of circulating virus may mask the presence of antibody. Numerous investigators have used the immune response to sheep erythrocytes (SRBC) to evaluate the immunocompetence of FV-infected mice (6, 10). These workers have demonstrated repeatedly that the response to this antigen in infected mice is severely depressed relative to normal levels.

In this report we describe preliminary investigations of immunological reactivity to SRBC in FV-infected and statolon-treated mice. Using the Jerne and Nordin assay for hemolytic antibody plaque forming cells (anti-SRBC PFC) (11), we found that statolon abrogates the FV-induced immunodepression of the anti-SRBC response.

Materials and Methods. Animals. Inbred female DBA/2J mice (Jackson Laboratory, Bar Harbor, ME) were employed in all experiments and weighed approximately 15–20 g. Food and water were supplied *ad libitum*.

FV. This was prepared as a 20% suspension of infected DBA/2 spleen in phosphate buffered saline and was originally obtained from Dr. Charlotte Friend. Following centrifugation (5000*g* for 1 hr) and filtration (through a 450 nm Millipore membrane filter) the virus preparation was frozen and stored at −70°. In all experiments FV was inoculated intraperitoneally at a dose of 1000–2000 LD_{50} (50% leukemia producing doses) in 0.2 ml/mouse.

Statolon. Supplied by Eli Lilly and Co., Indianapolis, IN (lot no. 354-1080B-220). Statolon was prepared for inoculation into mice in 1% sodium bicarbonate. The dose of statolon was 4 mg/mouse injected intravenously.

Assay for antibody plaque forming cells. Except where specifically noted all animals were immunized ip with 2×10^8 SRBC in normal saline 4 days prior to assay. Spleens were examined for IgM or IgG PFC against SRBC employing Jerne and Nordin's assay

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TABLE I. Effect of Statolon on the Anti-Sheep RBC Antibody Plaque Forming Cell Response of Normal and Friend Virus-Infected DBA/2 Mice.

Expt. no.[b]	Day of immunization[c]	Anti-sheep erythrocyte PFC $\times 10^5$ [a]/spleen			
		Control	FV	FV + statolon[d]	Statolon
I	−1	1.27 ± 0.29	0.43 ± 0.05	1.19 ± 0.50	0.96 ± 0.39
II	+1	0.71 ± 0.21	0.18 ± 0.10	0.72 ± 0.21	1.02 ± 0.33
III	+1	1.40 ± 0.31	0.34 ± 0.096	1.38 ± 0.28	2.28 ± 0.28
IV	+3	1.31 ± 0.30	0.15 ± 0.05	1.29 ± 0.50	3.05 ± 0.52
V	+3	1.04 ± 0.44	0.15 ± 0.07	0.95 ± 0.34	1.68 ± 0.57

[a] The mean number of antibody plaque forming cells $\times 10^5 \pm$ SD.
[b] Each experimental or control group consisted of at least 5 mice/group.
[c] 2×10^8 sheep RBC given ip on days relative to statolon administration.
[d] 4 mg of statolon/mouse given iv 3 days after 1000–2000 LD_{50} FV.

of localized hemolysis in gel (11).

Results. Experiments with sheep erythrocytes were conducted in order to examine the effect(s) of statolon on immunocompetence of FV-infected mice. FV was inoculated on Day 0 and 3 days later statolon was administered intravenously. On various days relative to statolon five mice from each of the four experimental groups were immunized with SRBC. Four days after immunization spleens were assayed for SRBC-antibody producing cells as described in Materials and Methods.

As shown in the five experiments illustrated in Table I, there was a consistent significant reduction in anti-SRBC PFC in FV-infected mice ranging from 67 to 90% of control values. The magnitude of this immunodepression was directly related to the length of the interval between FV inoculation and SRBC immunization. The FV-induced immunodepression could be abrogated by administration of statolon 3 days after virus inoculation with restoration of anti-SRBC PFC production in the spleens of all infected animals to normal control levels. Restoration of immunocompetence to normal levels in FV-infected mice by statolon, as measured by PFC response to SRBC, was demonstrable irregardless of when SRBC were injected with respect to statolon. In 2 of 4 experiments with uninfected mice, however, there was a significant increase in anti-SRBC PFC response over normal levels when SRBC were given 1–3 days after statolon.

One possible mechanism for the immunostimulatory effects of statolon may be a transient, low grade splenomegaly which follows statolon administration. This splenomegaly is accompanied by increases in the number of spleen cells. It is as yet unknown whether these additional cells arise *de novo* (in the spleen) or migrate from other areas (*e.g.*, the bone marrow). It is clear from Fig. 1, however, that they are composed of both antibody-forming and nonantibody-forming cells. This conclusion is based on the observation that whereas statolon treatment of uninfected mice results in increased numbers of anti-SRBC PFC in the whole spleen, the number of anti-SRBC PFC per 1,000,000 spleen cells remains constant.

Experiments were conducted to determine the effect of statolon on the kinetics of anti-SRBC PFC formation in FV-infected and uninfected mice. As in a previous experiment, statolon was administered 3 days after FV and SRBC 1 day later. Spleens were removed 2, 4, 8 and 12 days following immunization and assayed for IgM plaque forming cells. As shown in Table II, peak anti-SRBC PFC formation occurred 4 days after immunization in all four experimental groups; as in previous experiments, statolon treatment prevented or reversed immunodepression in FV-infected mice. In addition all spleens were assayed for the presence of anti-SRBC IgG PFC 8 and 12 days after immunization (Table II). Again statolon abrogated FV-induced immunodepression of the anti-SRBC PFC response.

Discussion. Administration of statolon to mice 3 days after Friend virus inoculation results in inhibition of virus replication and

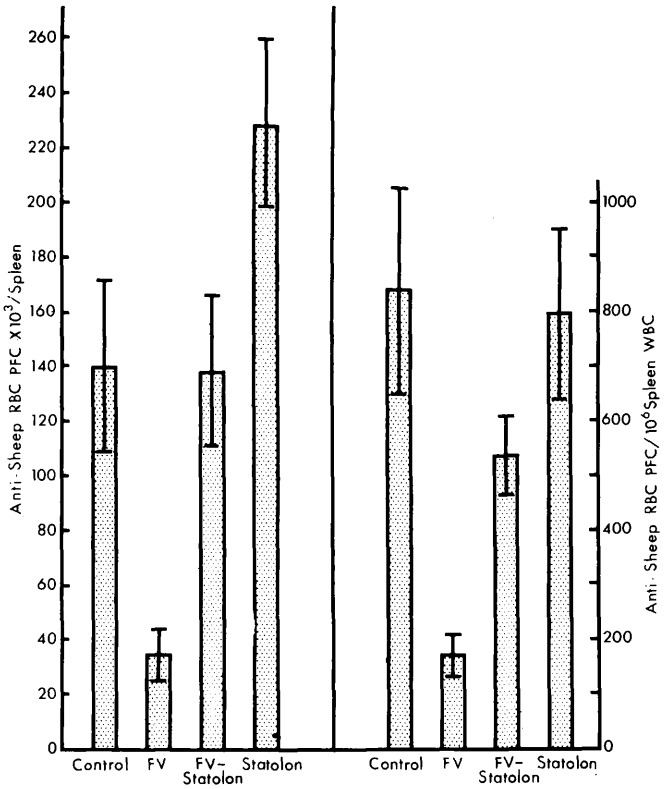

FIG. 1. Production of antibody plaque forming cells to sheep erythrocytes in spleens of normal, FV-leukemic and statolon-treated normal and leukemic mice. Statolon was administered 3 days after FV, sheep erythrocytes 1 day later and spleens were removed and assayed for antibody plaque forming cells on the fourth day after immunization. Brackets indicate one standard deviation. The number of PFC per spleen (left ordinate) is compared with the PFC per 10^6 splenic leukocytes (right ordinate).

conversion of a lethal infection into a dormant one. Suppression of the disease is preceded by the production of interferon and of antibody reactive against both virus and virus-transformed cells (4, 5). Since untreated leukemic mice are immunosuppressed in that they produce no virus-specific antibody, the presence of antibody in mice with dormant infections suggests that restoration of immunocompetence is a crucial step in the suppression of disease by statolon.

The present studies were undertaken to determine the extent of statolon's abrogation of FV-induced immunodepression. The diminished immune response of FV-leukemic mice to sheep erythrocytes has been used extensively as a measure of immunodepression in such mice. Our studies demonstrate that statolon abrogates this virus-induced immunologic depression. Statolon-treated, FV-infected mice produce normal numbers of both IgM and IgG PFC (see Tables I and II). In addition, the rate of PFC formation and the time of peak PFC formation in statolon-treated FV-infected mice is the same as in normal mice.

Statolon administered prior to SRBC consistently increased the PFC response in uninfected mice. This is in contrast to experiments of Pindak (12) who found that administration of statolon prior to mengovirus or chicken RBC (CRBC) depressed the humoral immune response to the virus but had no effect on the anti-CRBC response. Pindak indicated that the immunodepression noted with mengovirus occurred only after a single

TABLE II. Effect of Statolon on the Kinetics of Anti-Sheep Erythrocyte Antibody Plaque Forming Cell Production in Normal and Friend Virus-Infected Mice.

	Days after immunization[a] (mean PFC $\times 10^3 \pm$ SD)					
	2	4	8		12	
	IgM	IgM	IgM	IgM + IgG[b]	IgM	IgM + IgG
Control	2.6 ± 0.6	110 ± 30	7.2 ± 1.0	12.0 ± 2.0	4.0 ± 0.4	22.0 ± 3.8
FV	3.1 ± 0.8	32 ± 10	2.6 ± 0.6	3.0 ± 0.8	1.0 ± 0.8	2.8 ± 1.5
FV + Statolon[c]	4.4 ± 0.7	96 ± 20	6.6 ± 2.0	12.0 ± 1.0	4.2 ± 2.0	26.0 ± 8.0
Statolon	3.6 ± 0.6	130 ± 20	7.0 ± 2.0	9.0 ± 2.1	3.0 ± 0.6	16.0 ± 4.0

[a] All animals immunized with 2×10^8 sheep erythrocytes one day after statolon or 4 days after FV.

[b] For IgG, PFC, anti-IgG globulin (Meloy Labs.) diluted 1:500 in Hanks' BSS was added to each plate for 1 hr prior to the addition of complement. To determine number of IgG PFC, subtract IgM PFC from IgM + IgG PFC.

[c] 4 mg of statolon/mouse given iv 3 days after 1000–2000 LD_{50}, FV.

dose of statolon plus virus; the response following two consecutive doses of both statolon plus virus yielded antibody titers as high as those produced by a single dose of virus without statolon pretreatment. The depression of the humoral response to mengovirus after a single dose of statolon might be due to interferon production which would inhibit virus replication and thereby reduce the antigenic stimulus; the mice, however, would be primed by this exposure to virus and might respond optimally to the second exposure. In contrast, the magnitude of the antigenic challenge imposed by the "nonreplicating" CRBC would not be affected by statolon-induced interferon; normal and statolon-treated mice would be expected to respond equally on primary exposure to CRBC. However, with SRBC the "primary" PFC response was enhanced as a result of statolon treatment. The lack of enhancement in the CRBC system can be ascribed to differences in the doses of statolon employed. Alternatively, the differences noted might be explained by the sensitivities of the assays for immunoresponsiveness. The hemolytic assay for antibody producing cells, employed in the present investigation, is sensitive to 2-fold increases in cell number. However, in the hemagglutination titration, employed by Pindak, a 2-fold increase in antibody titer may only represent a difference of one tube usually not considered significant.

The increase in anti-SRBC PFC following statolon treatment of normal mice may be related to a transient hyperplasia of the spleen. This statolon-induced hyperplasia involves more than antibody-forming cells or their precursors since the number of anti-SRBC PFC per 10^6 spleen leukocytes remains constant following statolon treatment. It should be noted that the anti-SRBC PFC response in uninfected mice was enhanced by statolon whereas restoration to only normal levels was achieved in FV-infected mice. This difference could be explained by antigenic competition since many spleen cells in statolon-treated FV-infected mice may be in the process of making antibody to the virus at the time of sheep RBC injection.

A central question in the abrogation of FV-induced immunodepression is whether statolon prevents the development of immunodepression or restores immunocompetence by either acting directly on cells which are already immunodepressed or indirectly by inducing formation of a new population of immunocompetent cells. This question cannot easily be answered at present because of the prolonged time span involved in the development of immunodepression, in the action of statolon and in the anti-SRBC PFC assay for immunocompetence.

The most likely working hypothesis to explain the statolon-induced remissions in FV infections is that statolon induces interferon, thereby inhibiting viral replication, and either reverses immunodepression in infected

cells or stimulates the production of new spleen cells which by virtue of interferon are not subject to FV-induced immunodepression. These immunocompetent cells produce antibody to FV and FV-leukemic cells and thereby convert a lethal infection into a dormant one. They also are available for production of antibody toward other antigens, *e.g.*, the sheep erythrocyte.

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