Effect of Statolon on Aerogenic Immunization of Mice with Mengovirus-37A[1] (34894)

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The efficacy of interferon (IF) inducers in the prophylaxis and chemotherapeusis of certain virus infections has been shown experimentally. However, their effect in populations subjected to ongoing immunizations is questionable. Evidence accumulated to date indicates that the use of IF-inducers may be contraindictated with live virus immunization regimens. DeSomer *et al.* (1) reported that the administration of Sindbis virus or *E. coli* 24 hr before live poliovirus inhibited the synthesis of virus-neutralizing antibodies in rats. Pindak (2) observed that statolon-treated mice showed suppressed antibody formation after inoculation with the M variant of mengovirus.

Previously it was observed that mice exposed to immunogenic doses of avirulent mengovirus-37A produced demonstrable humoral antibodies and were resistant to lethal aerosol challenge doses of the antigenically similar Columbia-SK (Col-SK) virus (3). Thus with the mengovirus-37A model system used in conjunction with IF-inducers one could note the effect of induced IF synthesis on both the immune response and protection developed against lethal challenge doses of Col-SK virus.

The present study pertains to the effect of statolon on the aerogenic immunization of mice with mengovirus-37A, the delayed immune response observed, and the resultant diminution in protection against challenge with lethal aerosol doses of Col-SK virus.

Material and Methods. Viruses. Pools of Col-SK and mengovirus-37A were prepared in L-cells (L-929) and assayed as previously reported (4).

Aerosol exposure apparatus. The apparatus and related methodology has also been described (5).

Statolon. Statolon (kindly furnished by W. J. Kleinschmidt of the Lilly Research Laboratories) was dissolved in 1% sodium carbonate and then further diluted in Eagles' minimum essential medium (MEM) to give a final concentration of 1 mg of active ingredient in 0.2 ml.

Virus and interferon assay. At specified intervals after statolon administration or aerosol exposure six mice were killed and their lungs, spleen, and blood were collected. Each of the pooled specimens was weighed and homogenized with 9 vol of MEM. The suspensions were centrifuged and supernatant fluid assayed for virus content.

Serum samples were assayed for IF activity according to the method of Campbell and Colter (6). Infectious virus particles were inactivated prior to IF assay by adjusting the serum sample pH to 2.0 with 2 *N* HCL and holding at 4° for 18 hr before neutralizing with 2 *N* NaOH.

Serological procedures. The hemagglutination–inhibition (HAI), virus neutralization, and mercaptoethanol (2ME) degradation procedure have been described in a recent communication (3).

Results. The first series of experiments were designed to determine the effect of an intraperitoneal (ip) injection of 1 mg of statolon on aerogenic immunization with mengovirus-37A. Immediately after treatment (0 day), and at daily intervals of 10 days thereafter, 30 treated mice were randomly

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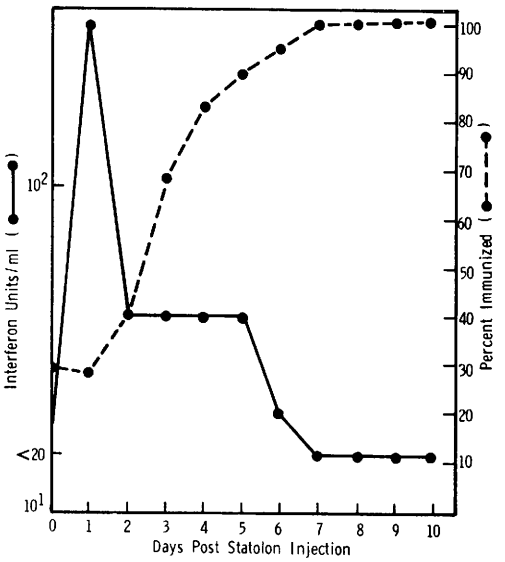

FIG. 1. Effect of a single injection of statolon on aerogenic immunization of mice with mengovirus-37A and resistance to lethal aerosol challenge doses of Col-SK virus. (a) Each point on interferon curve represents the titer of pooled sera of 10 statolon-treated mice. (b) Percent immunized as indicated at each point is based on the survival rate for groups of 20 mice.

selected (from the total of 330); 10 were exsanguinated and the collected sera assayed for IF activity, and 20 were exposed to immunogenic aerosol doses (6500 PFU/mouse) of mengovirus-37A. Twenty-one days later these same groups of mice were challenged with lethal aerosol doses (500 PFU/mouse) of Col-SK virus sufficient to kill 100% of normal control mice.

As shown in Fig. 1, aerogenic immunization and resistance to lethal virus challenge did not exceed 50% until the third day after statolon administration. By Day 4 however, resistance greater than 80% was observed. Serum IF levels peaked on the first day after statolon injection and dropped rapidly thereafter. By Day 7, little or no activity was detectable. One can conclude, based on resistance to virus challenge, that statolon administered ip up to 48 hr prior to mengovirus-37A aerosol exposure significantly reduced the number of mice immunized.

Accordingly, additional groups of mice were exposed to aerosols of mengovirus-37A on Days 0, 1, 3, and 5, after statolon administration. Hereafter, these will be referred to as groups 0, 1, 3, and 5. Mice from each group were bled and sacrificed on Days 0, 1, 6, 9, 12, 18, 21, and 31 after aerosol exposure. Serum IF levels HAI, and neutralizing antibodies were determined for each specified day. In addition, pooled blood, lung, and spleen tissues were assayed for virus content. The remaining animals in each group were challenged with lethal aerosol doses of Col-SK virus 21 days after aerogenic immunization.

In Fig. 2, groups 0 and 1, which received

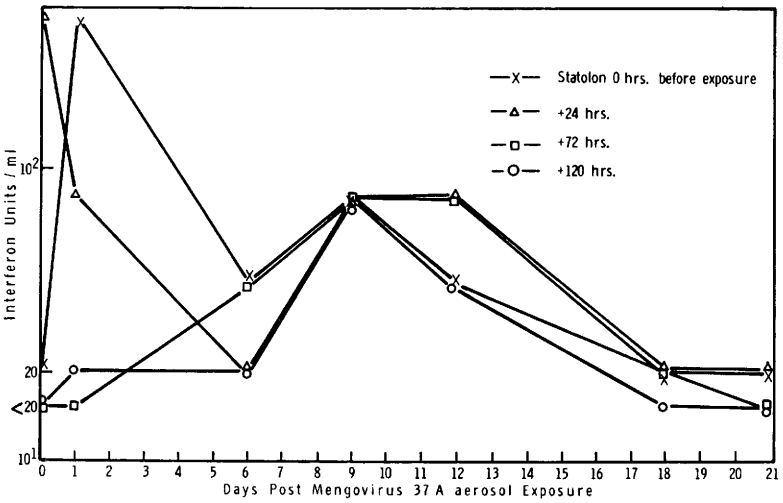

FIG. 2. Humoral interferon levels in statolon-treated mice exposed to mengovirus-37A aerosols. Each point represents titers from pooled sera of 40 mice.

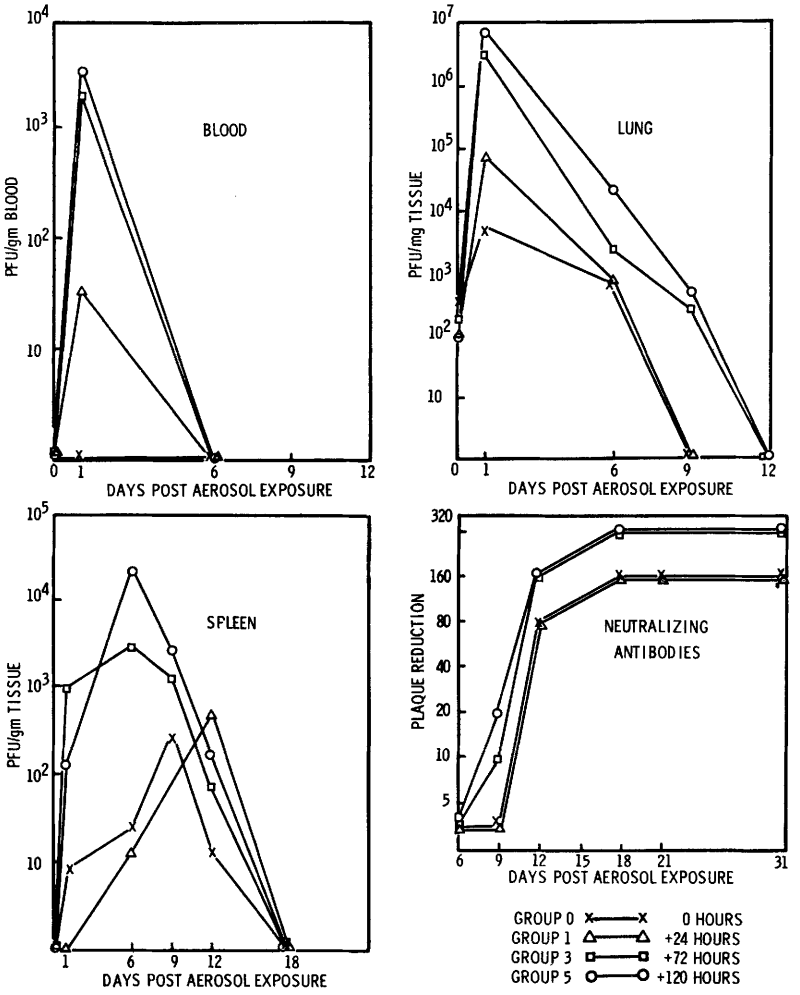

FIG. 3. Response of statolon-treated mice to aerosols of mengovirus-37A. Each point represents titers of pooled specimens of six mice.

statolon on the same day or 24 hr before aerosol exposure, demonstrated usual IF peaks at 24 hr after statolon administration which then dropped rapidly during the ensuing 24 hr. There was a second rise in IF activity (80 units) at Day 9 which was attributable to mengovirus-37A infection (3).

In groups 3 and 5 (which had received statolon 72 or 120 hr prior to immunogenic aerosol exposure) the IF humoral levels were 20 units/ml or less at the time of aerosol exposure and did not significantly rise until Day 9 when IF levels of 80 units were observed. It was apparent that the biphasic curve with reference to IF humoral activity was attributable to statolon and the subsequent virus infection.

The data in Fig. 3 show that group 0 mice evinced no viremia after mengovirus-37A exposure. Viremia was present, however, in the other groups (1, 3, and 5) on Day 1 after aerosol exposure and the titer for group 1 was significantly lower than that of groups 3 and 5.

Figure 3 also depicts the results of assaying lung suspensions for virus content. Immediately after aerosol exposure virus titers in lung tissue were the same for all groups. However, at 24 hr post exposure groups 3 and 5 exhibited "normal" virus titers whereas

TABLE I. Serologic Titers and Percent Mortality after Col-SK Challenge of Statolon-Treated Mice 21 Days After Immunization with Mengovirus-37A.

Statolon-treated groups[a]	HAI titers[b]	Neut. titers[c]	No. challenged with lethal Col-SK aerosol doses	Percent deaths
0	16	160	60	70
1	32	160	60	72
3	64	320	60	31
5	128	320	60	6.5
Immunized controls (37A only)	256	160	120	4
Normal controls	<2	<2	120	99

[a] Days after statolon injection on which mice were exposed to mengovirus-37A aerosols.

[b] Reciprocal of highest dilution which inhibited hemagglutination (pooled sera of 40 mice per group).

[c] Reciprocal dilution of serum that resulted in 50% reduction in the number of plaque-forming units of virus (pooled sera of 40 mice per group).

group 0 and 1 were 1 to 2.5 logs lower. Spleen tissues assayed for virus content revealed that groups 3 and 5 were similar to untreated controls and reached peak titers at 6 days, whereas groups 0 and 1 had lower titers and peaked later (9–12 days). One can conclude that statolon administered prior to aerogenic immunization with an attenuated virus significantly reduces the amount of virus in the lungs, spleen, and blood.

Neutralizing antibodies appeared by Day 9 for groups 3 and 5 only (Fig. 3). It was apparent that in groups 0 and 1 there was not only a delay in antibody response but the titers attained did not reach the levels observed with groups 3 and 5. It is also of interest to note that at Day 9 when humoral neutralizing antibodies were present in groups 3 and 5 there were still significant quantities of virus present in the lungs.

Similar to results observed with regard to neutralizing antibodies, groups 0 and 1 in comparison with groups 3 and 5 exhibited a delayed HAI response (not shown in Fig. 3) as well as lower antibody titers.

Treatment of whole sera collected from groups 1, 3, and 5, with 2-ME revealed that Day 9 neutralizing and HAI activity was 2-ME-sensitive. Day 12 specimens showed only a slight reduction in titers after treatment, whereas 21-day antibodies were resistant. This was essentially the same as was seen in the mengovirus-37A immune response in untreated control mice (3).

Table I depicts the serological results observed at 21 days post aerogenic immunization with mengovirus-37A and the degree of protection produced against challenge with lethal aerosol doses of Col-SK virus. It is not possible to correlate antibody titers with resistance in this case. The differences in neutralizing antibody titers were not significant, yet the death rates ranged from 70% (group 0) to as low as 6.5% (group 5).

Discussion. In a previous study it was observed that 1 mg of statolon injected ip 24 hr prior to exposure with lethal aerosol doses of Col-SK virus induced a resistance 60 times greater than that of normal mice (7). The treated mice which survived showed no immunity or resistance to a second lethal aerosol exposure 21 days later.

Similar to the above findings, 1 mg of statolon injected ip into mice 24 or 48 hr before avirulent mengovirus-37A aerosol exposure reduced the number of mice resistant to subsequent lethal aerosol challenge to less than 30%. When mice were exposed to mengovirus-37A aerosols on the third day post statolon treatment, over 70% survived lethal challenge doses of Col-SK virus. Therefore, there was a very restricted time period (approximately 48 hr) after statolon treatment in which the administration of the IF inducer

could be shown to significantly effect the aerogenic immunization of mice with mengovirus-37A. Regardless of the differences in IF levels prior to mengovirus-37A exposure, all groups (0, 1, 3, and 5) by Day 9 post exposure exhibited similar levels of IF activity. There was also an inverse correlation between humoral IF and virus levels at 24 hr: (1) group 0, 320 units of IF activity and no detectable viremia; (2) group 1, 80 IF units/ml serum and 15 PFU/gram of whole blood; (3) groups 3 and 5, 20 or less IF units and viremia exceeding 10^3 PFU/g. IF levels could also be associated with differences in the virus titers of lung and spleen samples.

One of the most significant findings of the study was that statolon treatment did not entirely suppress humoral antibody formation. DeSomer *et al.* (1) reported that mice injected intravenously with *E. coli* and vaccinated 24 hr later with poliovirus, produced only negligible titers of neutralizing antibodies. The fact that we observed an antibody response in statolon-treated mice may be attributed to mengovirus-37A replication in the mouse in contrast to the nonreplicating antigen employed by DeSomer *et al.* (1).

Pindak (2) reported that statolon-treated mice inoculated with the avirulent M variant of mengovirus exhibited reduced neutralizing-antibody titers at 14 days. We also observed reduced neutralizing-antibody titers in groups 0 and 1 at 12 days post mengovirus-37A exposure but by Day 18 the titers had increased almost to the levels achieved by groups 3 and 5 and the normal controls. The differences between Pindak's observations with the M variant of mengovirus and ours with mengovirus-37A may be attributed to the inherent characteristics of the two virus strains as well as differences in the route of introducing the immunogens; *i.e.*, ip versus aerosol.

The correlation of humoral antibody levels with resistance to lethal Col-SK aerosol challenge was not consistant. For example, the neutralizing-antibody titers were not significantly different for all groups studied and yet resistance to lethal challenge varied indicating that some other factor besides humoral antibodies was influencing resistance to respiratory challenge. Although 19S and 7S antibodies were observed in serum after aerogenic immunization of mice with mengovirus-37A (3) possible IgA activity in the respiratory tract secretions remains unknown. This problem needs further investigation, as Fazekas de St. Groth and Donnelley (8) reported that bronchial rather than serum antibodies were more important for protection against challenge with virulent influenza virus. This is also in accordance with the recent report of Ogra *et al.* (9) who administered inactivated poliovirus vaccine to children intranasally and noted a secretory IgA response in the absence of any significant serum IgA response.

This leads one to conclude that statolon administration can indeed influence the mengovirus-37A immune response of mice and resistance to lethal aerosol challenge doses of Col-SK virus.

Summary. A single injection of statolon administered prior to aerogenic immunization with mengovirus-37A resulted in reduced amounts of virus in lungs, spleen, and blood of treated mice and a decrease in resistance to lethal aerosol doses of the antigenically similar Columbia-SK virus. There was a delay as well as diminution in antibody response which, along with decreased virus titers, could be associated with serum interferon levels. Mice treated with statolon prior to aerogenic immunization and later subjected to lethal aerosol challenge exhibited resistance which was not consistant with humoral antibody levels.

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