



Alteration by Concanavalin A or silica of the immune response of mice to polyvinylpyrrolidone
by Dennis Earl Bier

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE
in Microbiology

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Abstract:

The regulation of the immune response to the thymus independent antigen polyvinylpyrrolidone (PVP) was investigated using Concanavalin A (Con A), a specific T cell mitogen. Administration of 150 μ g of Con A given i.v. before or at the time of immunization with PVP significantly suppressed the PFC response to PVP in normal mice but not in nude mice. Con A given 2 days after immunization resulted in a 5-10 fold increase in PVP-specific PFC in normal mice but not in nude mice. These results indicated that amplification and suppression of the PFC response to PVP were thymus dependent. Kinetic studies revealed that Con A induced suppression was evident by day 4 of the response to PVP and reached maximal proportions by day 5 while Con A induced amplification was not evident until day 5. Con A given 2 days after immunization with PVP completely reversed the effects of low-dose paralysis to PVP.

The role of macrophages in in vivo antibody responses to PVP, a thymus independent antigen, was investigated by the use of silica, a specific macrophage toxin. Silica (2.5 mg) was given i.v. at various times with respect to immunization with PVP produced a biphasic amplification of the PVP-specific PFC response. The PFC response was further amplified (6-10 fold) when silica was given on both peaks of amplification of the biphasic curve. Silica produced significant amplification of the PFC response when given to normal mice but not in nude mice indicating that the amplification was thymus dependent. Silica partially reversed the suppression induced either by Con A or in mice paralyzed by a low-dose of PVP.

Collectively, these results indicate that the magnitude of the immune response may be regulated by thymus-derived lymphocytes.

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RESPONSE OF MICE TO POLYVINYLPYRROLIDONE

by

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A thesis submitted in partial fulfillment
of the requirements for the degree

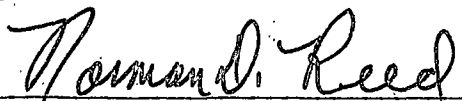
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ABSTRACT

The regulation of the immune response to the thymus independent antigen polyvinylpyrrolidone (PVP) was investigated using Concanavalin A (Con A), a specific T cell mitogen. Administration of 150 μ g of Con A given i.v. before or at the time of immunization with PVP significantly suppressed the PFC response to PVP in normal mice but not in nude mice. Con A given 2 days after immunization resulted in a 5-10 fold increase in PVP-specific PFC in normal mice but not in nude mice. These results indicated that amplification and suppression of the PFC response to PVP were thymus dependent. Kinetic studies revealed that Con A induced suppression was evident by day 4 of the response to PVP and reached maximal proportions by day 5 while Con A induced amplification was not evident until day 5. Con A given 2 days after immunization with PVP completely reversed the effects of low-dose paralysis to PVP.

The role of macrophages in in vivo antibody responses to PVP, a thymus independent antigen, was investigated by the use of silica, a specific macrophage toxin. Silica (2.5 mg) was given i.v. at various times with respect to immunization with PVP produced a biphasic amplification of the PVP-specific PFC response. The PFC response was further amplified (6-10 fold) when silica was given on both peaks of amplification of the biphasic curve. Silica produced significant amplification of the PFC response when given to normal mice but not in nude mice indicating that the amplification was thymus dependent. Silica partially reversed the suppression induced either by Con A or in mice paralyzed by a low-dose of PVP.

Collectively, these results indicate that the magnitude of the immune response may be regulated by thymus-derived lymphocytes.

INTRODUCTION

"A slow sort of country!" said the Queen. "Now, here, you see, it takes all the running you can do, to keep in the same place. If you want to get somewhere else, you must run at least twice as fast as that."

Through the Looking Glass
Lewis Carroll

The successful induction of humoral immune responses to most antigens appears to require collaboration among at least three distinct cell types: bursa-equivalent lymphocytes (B cells), thymus-derived lymphocytes (T cells), and adherent or accessory cells (macrophages) (1). Antigens requiring such collaboration characteristically are complex, present multideterminant features, and are strictly dependent on the presence of functional T cells for specific immune responses (1); i.e. they are thymus dependent (TD) antigens. However, a select group of antigens can elicit specific antibody responses in the apparent absence of T cells and, therefore, are termed thymus-independent (TI) antigens (2). Some of these TI antigens have met stringent criteria by stimulating spleen cells from congenitally thymus-deficient (nude) mice to produce antigen-specific antibody either in vitro or in vivo. These TI antigens include: dinitrophenylated polymerized flagellin (DNP-POL) (3), Vi (4), lipopolysaccharide (LPS) (5), type III pneumococcal polysaccharide (S-III) (5), and polyvinylpyrrolidone (PVP) (6). The basic structure of all TI antigens is similar; they are all polymers of

repeating antigenic determinants and show prolonged antigen persistence in the host (7).

TI antigens have been reported to be macrophage independent (8). However, some controversy has developed regarding this concept. Recent evidence suggests that TI antigens may require a qualitatively and quantitatively different macrophage population than TD antigens (9-11). These differences may become apparent only when different methods of macrophage depletion are used (9-11), and may also depend on the specific TI antigen (12).

T cells are responsible for mediating a variety of immune phenomena. Graft vs. host reactions (13), parasite elimination (14,15), and helper (16) and suppressor (17) effects on antibody production are thymus-dependent events. This diversity of function suggests that T cells are a heterogeneous population whose functions are genetically predetermined. Accordingly, many of these functions are tentatively assigned to definite T cell subpopulations according to the type(s) of cell surface determinant(s), called Ly antigens, found on mouse T cells. For example, T cells which are preprogrammed to help B cells in antibody production are phenotypically $\text{Ly-1}^+2,3^-$. Both cytotoxic effector cells and suppressor cells implicated in regulating antibody production have $\text{Ly-1}^-2,3^+$ surface antigens (for review see 18), but, identification on suppressor cells of antigens of the I region of the H-2 histocompatibility complex of mice differentiates these cells from

cytotoxic cells (19).

Effects produced by these subclasses of T cells can be either specific or nonspecific depending on the stimulus. Cantor et al. showed specific helper and suppressor effects after priming the appropriate Ly subpopulation with the antigen sheep red blood cells (20). Stimulation of T cells in mixed lymphocyte culture will produce a non-specific helper T cell replacing factor, called allogeneic effect factor (AEF) (21), the production of which may be dependent on the presence of $\text{Ly-1}^{+2,3-}$ T cells (18). Concanavalin A (Con A), a plant lectin derived from the jackbean, is a polyclonal activator of T cells only (22). Exposure of $\text{Ly-1}^{+2,3-}$ and $\text{Ly-1}^{-2,3+}$ T cells to Con A non-specifically activated these T cell subpopulations to exert their respective helper and suppressor effects (23), perhaps without the requirement for DNA synthesis (24).

Suppressor cells in particular are receiving a great deal of attention because they offer another possibility for selectively manipulating immune responses. Suppressor cells have been implicated in regulating both high and low zone tolerance (25,26), antigenic competition (27), allotype and idiotype suppression (28,29), and IgE antibody production (30). Various immunodeficiency disorders have been associated with suppressor cells. For example, immunoglobulin synthesis was limited by suppressor cells in some forms of common variable hypogammaglobulinemia (31). Peripheral blood lymphocytes from patients

with anergy associated with fungal infections were found to suppress T cell mitogenic and antigenic responsiveness (32). The decreased number of hemopoietic stem cells in Diamond-Blackfan syndrome was found to be associated with suppressor lymphocytes (33).

Just as unwarranted suppressor cell activity may be disadvantageous, so may the loss of suppressor activity. Evidence indicates that loss of effective suppression may lead to various forms of autoimmunity (34). The most commonly used animal model in studies of autoimmunity has been the New Zealand Black mouse. These mice are prone to the development of spontaneous autoimmune syndrome similar to systemic lupus erythematosus (35). The difficulty in establishing tolerance (36) and hyperproduction of antibodies against some antigens (37) in these mice may be explained by a decreased function of suppressor cells. This decline in suppressor cell activity is age related both in New Zealand Black mice (38) and other normal mouse strains (39).

The demonstration of regulation of immunity by suppressor T cells was initially shown in a system using the TD antigen sheep red blood cells (40). However, Baker and his coworkers showed that the magnitude of the antibody response to S-III, a TI antigen, also may be regulated by T cells (41). Administration of antilymphocyte serum (ALS), a T cell depleting drug, produced enhancement of the antibody response to S-III which could be abrogated by infusion of syngeneic thymocytes (42).

In contrast, ALS-treated mice given syngeneic peripheral blood lymphocytes showed additional enhancement of the antibody response to S-III (42). On the basis of these and subsequent observations, Baker has hypothesized that two thymus-dependent cells, termed suppressor and amplifier cells, act in an opposing manner to regulate the magnitude of the antibody response to S-III (43). As additional evidence for the thymus dependency of suppressor and amplifier cells, ALS treatment of nude mice does not result in enhancement when compared with control mice (44). This observation also discounts any mitogenic effect ALS may have on B cells (44).

Kerbel and Eidinger showed that ALS treatment of mice will enhance antibody production to PVP, also a TI antigen (45). Adult thymectomy increased the antibody response to PVP. The increase was partially reversed by thymus implantation or by injections of hydrocortisone-resistant thymocytes (46). PVP has been observed to induce antigen-specific low zone paralysis through a mechanism possibly involving suppressor T cells (47,48). This mechanism may be analogous to the low zone paralysis induced by S-III (49).

Nonspecific activation of T cells by plant lectins has enabled investigators to more closely study various cell interactions. The effects of Con A on in vitro antibody production were extensively investigated by Rich and Pierce (50). These investigators have shown that incubation of spleen cells with Con A causes the release of a

soluble product(s), called soluble immune response suppressor (SIRS), which nonspecifically suppresses in vitro antibody responses to both TD (51) and TI (52) antigens. The production of SIRS is T cell dependent (53). SIRS has the macrophage as its target cell (54). Con A induced enhancement of in vitro antibody responses was also observed and was found to be dependent on the appropriate culture conditions and time of Con A addition (55).

Treatment of mice in vivo with Con A was observed to suppress skin graft rejection (56), heart allograft rejection (57), delayed hypersensitivity reactions (58), and helper T cell dependent antibody responses (59), if the lectin was administered before or at the time of immunization. Other studies have shown that Con A may enhance in vivo humoral (60,61) and cell-mediated immune responses (62).

Very recently, Baker and coworkers have shown that Con A treatment of mice can either suppress (63) or enhance (64) antibody responses to S-III depending on the time of Con A administration with respect to immunization. Baker interpreted these results to indicate that Con A treatment at the time of immunization activated suppressor cells which may have acted on both amplifier T cells and B cells (63). Con A given after immunization with S-III may have activated amplifier cells which may subsequently have enhanced the antibody response (64).

Macrophages are believed to play a fundamental role in antibody response to TD antigens (1). Their role in antibody responses to TI

antigens is less clear (8-12, 65). One method of macrophage removal involves treatment of macrophages either in vivo or in vitro with various macrophage toxins. Silica has been reported to be a selective macrophage toxin (66). Its toxic activity has been attributed to its ability to bind to lipoproteins present in the macrophage lysosomal membrane. This binding disrupts the integrity of the membrane allowing leakage of lysosomal enzymes into the cell cytoplasm which results in cell lysis (67).

Silica has been used as an aid in identifying host defense mechanisms to a variety of organisms. It enhances the susceptibility of mice to certain viral (68), parasitic (69), and bacterial infections (70,71). Silica can reduce host immunity to tumors by interfering with the establishment of active (72) and adoptive (73) immune mechanisms. A variety of neoplasms are linked directly to silica exposure (74).

Pearsall and Weiser reported that intraperitoneal administration of silica prolonged skin allograft rejection when given before or after skin grafting (75). Reversal of such prolongation was achieved by treatment of animals with poly-1-vinylpyridine N-oxide, a macrophage

stabilizing agent (76). Abrogation of genetic resistance¹ by silica will allow takes of allogeneic and even xenogeneic bone marrow grafts in recipients (77).

In vitro, ingested silica particles produced distinct morphologic and biochemical changes in macrophages (78,79). Levy and Wheelock found that intravenously administered silica reduced the in vitro cytolytic response of spleen cells from mice previously challenged with histoincompatible cells in vivo (80).

Inhalation of silica dust in mice enhanced splenic T cell responsiveness to Con A, but simultaneously reduced B cell immunocompetence (81). Aerosol exposure of animals to silica depressed the ability of antigen-primed alveolar and splenic macrophages to serve as accessory cells in initiating antibody forming cells in irradiated hosts (81) and to phagocytize bacteria (81).

¹ Genetic resistance may be defined as an increase in the number of bone marrow cells needed for survival of an irradiated recipient as the number of histocompatibility differences between bone marrow donor and recipient. Major histocompatibility differences are a prerequisite for resistance although additional genetic factors influence the outcome. These additional factors appear to be located outside the major histocompatibility complex in mice but may be contained within the major histocompatibility complex in dogs.

Silicosis in humans and experimental animals is associated with an increase in circulating abnormal antibodies (82) and with autoimmune diseases such as rheumatoid arthritis (83). Schuyler et al. found no differences between silicotic patients and control groups when lymphocyte responsiveness, peripheral blood lymphocyte counts, or delayed hypersensitivity tests were compared (84). There is apparently no association between HLA phenotype and predisposition to silicosis (85).

Silica has been shown to have adjuvant effect on antibody production. Pernis and Paronetto (86) demonstrated that intravenously administered silica produced a marked increase in antibody production to ovalbumin. The antibody titer was higher when the interval between silica and antigen injection was longer. They hypothesized this increase was due to nonspecific stimulation of the reticuloendothelial system (86). Spitznagel and Allison found that silica enhancement of the antibody response to bovine serum albumin was comparable to that produced by lipopolysaccharide (87). Adsorption of antigen on the silica particles was not required for its adjuvant effects (88). Silica may also enhance antibody responses to particulate antigens (88) as well as to the aforementioned soluble antigens (86).

The objectives of this study were:

- 1) to determine the effects of Con A on regulatory mechanisms to PVP,

2) to determine the role of macrophages in the immune response to PVP.

MATERIALS AND METHODS

Animals

Balb/c mice raised at this laboratory were maintained on sterilized Wayne Lab Blox (Allied Mills, Inc., Chicago, IL) and acidified-chlorinated water ad libidum. Congenitally thymus-deficient (nude) mice and their phenotypically normal littermates (NLM: nu/+ or +/+) were derived from a colony in which cross-intercross mating is in progress to obtain a line of NLM and nude mice congenic with Balb/c mice. Some nude mice and NLM were obtained from a colony of Balb/c mice in which cross-intercross mating has produced a line of NLM and nude mice congenic with Balb/c mice. The breeding stock for these mice was originally purchased from Bomholtgard Ltd., Ry, Denmark. Experiments using these mice have been appropriately identified.

Animals of either sex were used and ranged in age from 6-12 weeks at the start of all experiments.

Antigens and Immunizations

Polyvinylpyrrolidone K90 (PVP) used for immunization or tolerance induction was donated by GAF Corporation, New York, NY, and had an average molecular weight of 360,000 daltons. All injections of PVP were given via the lateral tail vein in 0.25 ml of phosphate buffered saline (PBS).

Type III pneumococcal polysaccharide (S-III) was donated by Dr. Phillip J. Baker, Bethesda, MD. The immunologic properties of

the S-III and the method by which it was prepared have been described (89-92). For immunization of mice, 0.5 μ g of S-III was administered as a single intraperitoneal (i.p.) injection in 0.5 ml of physiological saline.

Lipopolysaccharide (LPS) was donated by Dr. Jon A. Rudbach, University of Montana, Missoula, MT. The LPS was extracted from Escherichia coli 0113 (Braude strain) by the phenol-water procedure (93). Its preparation and properties have been described (94). For immunization of mice, LPS was administered as a single intravenous (i.v.) injection of 10 μ g in 0.10 ml of PBS.

Sheep red blood cells (SRBC) were obtained from Colorado Serum Co., Denver, CO. Mice received a single i.v. injection of 0.25 ml of a 4-times washed 10% suspension of SRBC in PBS.

Concanavalin A

Concanavalin A (Con A) batch #3212, carbohydrate content <0.1%, was purchased from Pharmacia Fine Chemicals, Piscataway, NJ. It was dissolved in PBS and given i.v. in 0.25 ml at the times and doses designated in the Results section.

Detection of Antibody-Forming Cells

Plaque-forming cells (PFC) specific for PVP, S-III, LPS, or SRBC were detected by a slide modification (95) of the localized hemolysis in gel technique (96). SRBC, or SRBC coated with LPS (97), PVP (98),

or S-III (99) were used as indicator cells. PFC responses to SRBC, PVP, and S-III were determined 5 days following immunization with the appropriate antigen. LPS responses were determined 4 days following immunization.

To quantify LPS-specific PFC, SRBC were coated with LPS from E. coli 0113 by the method of Neter et al. (97). Briefly, SRBC were washed 3 times in PBS. Washed cells were incubated for 30 min at 37°C in a 1:10 dilution in PBS of a 1 mg/ml solution of boiled (2.5 hrs) LPS per 0.5 ml of packed cells. These cells were then washed 3 times in PBS and a 10% suspension was used for the assay.

The number of PVP-specific PFC was determined using the modification of Lake and Reed (98) of the method originally described by Rotter and Trainin (99). SRBC were washed 3 times and resuspended to a 5% suspension in PBS. Equal volumes of washed cells and a previously aged (10-12 hours) tannic acid solution (0.1 mg/ml in PBS) were incubated at room temperature for 15 min. The tanned SRBC were divided into 2 equal portions and washed 3 times in PBS. One portion of the cells was resuspended to a 1:11 suspension in PBS to serve as the SRBC control in the plaque assay. The other portion was resuspended to a 5% concentration in PBS. Equal volumes of the 5% cell suspension and a 0.1 mg/ml PVP solution (K & K Laboratories, pharmaceutical grade) were incubated at room temperature for 15 min. The PVP-coated SRBC were washed 3 times

in PBS and resuspended in PBS to a 1:11 suspension for use in the plaque assay.

SRBC were coated with S-III by the chromium chloride coupling method of Baker et al. (100). SRBC were washed 4 times in saline and 0.5 ml of washed, packed SRBC was added to a centrifuge tube. One ml of a 1:10 dilution of a 1% solution of chromium chloride in saline followed by 1 ml of a 1 mg/ml S-III solution in saline were added to the packed cells. The resultant suspension was mixed and incubated at room temperature for 5 min. The S-III coated cells were washed 4 times in saline and resuspended to a 10% suspension in saline for use in the plaque assay. The uncoated, washed SRBC were resuspended in saline to a 10% suspension for use as SRBC control.

PVP Passive Hemagglutination Assay

Serum antibodies specific for PVP were determined by a microtiter assay (101). SRBC were coated with PVP as previously described. Coated cells were resuspended to a 0.25% suspension in PBS containing 0.4% gelatin were added as diluent to each well of a V-bottom microtiter tray. Two-fold serial dilutions of serum were made in the diluent and twenty five microliters of coated cells were added to each well. The tray was agitated and incubated at room temperature for 4-10 hours before patterns were read. The endpoint was judged to be the last well showing any agglutination when compared with control

wells.

Silica Particles and Preparation of Silica Suspensions

Crystalline silica particles, Min-U-Sil #216, (Whittaker, Clark, and Daniels, Inc., South Plainsfield, NJ) of less than 5 μm diameter were donated by Dr. Jon A. Rudbach, University of Montana, Missoula, MT, and were prepared using the method of Larson (102) by me or by Drs. Jim Cutler and Ken Lee of this institution. Briefly, 50 g of silica were suspended in 300 ml of distilled water and were exposed for 3 min of ultrasonic vibration in a Sonogen Model D-50 ultrasonic cleaner (Branson Instruments, Inc., Stanford, CT). This suspension was poured into a 1 l graduated cylinder, diluted with distilled water to a volume of 1 l, inverted several times and allowed to settle at room temperature for 24 hours. Portions of 250 ml each of the suspension were then aspirated into separate centrifuge tubes. The 500-750 ml portion from the top, designated Fraction III, was washed 3 times at 200 x g in distilled water, and poured into a petri dish and dried for 48 hours in a 60°C oven. Larson determined by optical measurement that the fraction III consisted of particles of the following sizes: 2.1 μm X 2.8 μm (80%), 1.4 μm X 3.5 μm (10%), and 1.4 μm X 2.1 μm (10%) (102).

Standard reference silica (103) DQ12<5 μm was a gift of Dr. Klaus Robock, Neuss, Germany, and was used to compare the effects on the immune response of fraction III Min-U-Sil.

All silica powders were autoclaved before use. In some experiments, the silica powders were baked for 4 hours at 180°C in an effort to render them pyrogen free (104). No effort was made to determine if this baked silica was indeed pyrogen free. Prior to injection, the silica was weighed, diluted with sterile PBS in a sterile container, and sonicated for 3 min. All doses of silica were administered either i.v. or i.p. in 0.5 ml.

Carbon Clearance Assay

The phagocytic index of the reticuloendothelial system (RES) of normal and silica-treated mice was assessed by measuring the ability of mice to clear carbon from their blood. Carbon clearance was determined using the modification of Levy and Wheelock (80) of the Biozzi et al. technique (105). Mice were weighed, bled from the retro-orbital plexus, and injected i.v. with 0.01 ml of a 10 mg/ml colloidal carbon (Pelikan ink, Gunther Wagner, Hanover, Germany) in 1% gelatin solution per gram body weight. Three min and 15 min after injection of the carbon, mice were bled from the orbit. At each bleeding 0.025 ml of blood was lysed in 4.0 ml of 0.1% Na₂CO₃. Optical densities (O.D.) were determined in a Varian Techtron Model 635 spectrophotometer at 650 nm. The phagocytic index, K, was determined for each mouse by the formula (105):

$$K = \frac{\log_{10} \text{O.D. } 3 \text{ min} - \log_{10} \text{O.D. } 15 \text{ min}}{12 \text{ min}}$$

The phagocytic index was corrected for differences in body and organ weight, α , by the formula (105)

$$\alpha = \sqrt[3]{K \times (\text{liver} + \text{spleen weight}) / \text{body weight}}$$

Statistics

Results are presented as the arithmetic mean PFC/spleen or PFC/ 10^6 spleen cells $\pm$ standard deviation for similarly treated groups of mice. Differences between values were judged to be significant when p values < 0.05 as assessed by Student's t test (106).

RESULTS

Effect of different doses of Con A on the PVP-specific PFC response

Previous experiments had established that Con A treatment of mice at the time of immunization suppressed the PFC response to PVP, while Con A treatment 2 days after immunization amplified the response (48). Because of the toxicity of Con A for mice, it was decided a more accurate assessment of the dose effects of Con A should be made by determining the effects of Con A amplification of the PVP-specific PFC response rather than suppression.

To determine the dose effects of Con A, Con A was given in doses of 75, 150, or 300 μg 2 days after immunization with 0.25 μg PVP, and the number of PFC generated was determined 5 days after immunization. Table 1 shows that all doses of Con A amplified the number of PFC generated; 150 μg produced the greatest increase in PFC. This amount of Con A was considered to be the optimal dose and was used subsequently to induce both suppression and amplification of PFC responses to PVP.

Effect of giving Con A at different times with respect to immunization with PVP

Previous studies had shown that the time of Con A administration with respect to immunization resulted in either suppression or amplification of the PFC response to both TD (51) and TI (52) antigens. Therefore, it was important to determine how Con A affected the PFC

TABLE 1. EFFECT OF DIFFERENT DOSES OF CON A ON THE PVP-SPECIFIC PFC RESPONSE.

DOSE OF CON A ^a	PFC/SPLEEN ^b	MORTALITY
		SURVIVORS/RECIPIENTS ^c
0	18,865 ± 6,190	5/5
75	121,555 ± 25,128	5/5
150	133,515 ± 16,186	5/5
300	48,363 ± 37,565	2/6

^aCON A WAS GIVEN I.V. 2 DAYS AFTER IMMUNIZATION WITH PVP.

^bARITHMETIC MEAN ± S.D. OF THE NUMBER OF PVP-SPECIFIC PFC DETERMINED 5 DAYS AFTER IMMUNIZATION WITH 0.25 µg PVP.

^cMORTALITY WAS DETERMINED BY DEATH BETWEEN 24 AND 72 HOURS AFTER INJECTION OF CON A.

response to PVP when given at various times with respect to immunization.

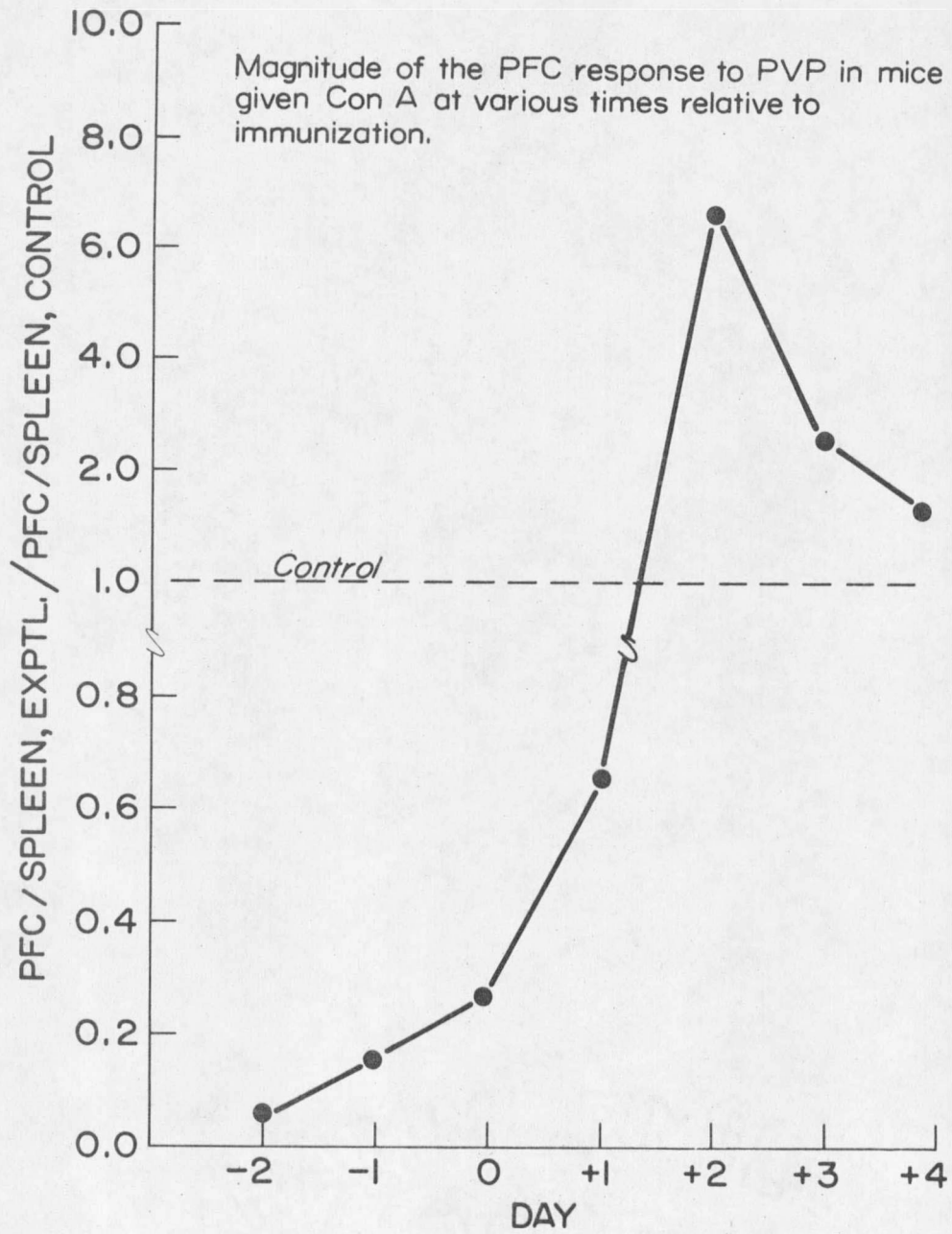
Groups of NLM (Bomholtgard Ltd., Ry, Denmark) were given a single injection of 150 μ g of Con A on days -2, -1, 0, +1, +2, and +3 with respect to immunization with PVP. All mice received the optimal immunogenic dose of PVP (0.25 μ g) on day 0 and the number of PVP-specific PFC was determined 5 days later. Figure 1 shows that groups injected with Con A on days -2, -1, 0 or +1 were suppressed. Amplification of the PFC response to PVP occurred when Con A was given on days +2, +3, or +4; the greatest amplification was observed when Con A was given on day +2. Con A was routinely given subsequently on day 0 to suppress PFC responses to PVP or on day +2 to amplify responses.

Effect of Con A on the PVP-specific PFC response of nude and normal mice

Previous reports suggested that the immune response to PVP may be regulated by suppressor T cells (6). Rich and Pierce showed that Con A treatment of spleen cells in vitro induced the development of non-specific suppressor T cells (53). Recently Markham et al. (63,64) presented evidence indicating the necessity of T cells for in vivo suppression or amplification of the PFC response to S-III.

To determine the thymus dependence of Con A suppression or enhancement of PFC response to PVP, groups of nude mice and their NLM

Figure 1. Magnitude of the PVP-specific PFC response in mice given Con A at different times with respect to immunization. Mice were given 150 μ g Con A i.v. at various times with respect to immunization with 0.25 μ g PVP on day 0. Each point represents the mean PFC of 5 mice generated 5 days after immunization with PVP.



(Bomholtgard Ltd., Ry, Denmark) were given 150 μ g Con A either on day 0 or day +2 with respect to immunization. All mice received the optimal immunogenic dose of PVP (0.25 μ g) on day 0 and their PFC values were assessed on day 5. The data of Table 2 show that Con A treatment of NLM either suppressed or amplified the PFC response depending on the time of Con A administration with respect to immunization. In contrast, Con A-treated nude mice showed neither marked suppression nor amplification of the PVP-specific PFC response. These results suggested the thymus dependency of Con A-induced suppression or amplification of the PFC response to PVP. The Con A-induced suppression or amplification of PFC responses to PVP may have been due to activation of suppressor and amplifier T cells respectively.

Kinetics of the Con A-induced amplification of the PVP-specific PFC response

Kinetic studies by Markham et al. (64) showed that Con A induced amplification of the PFC response to S-III was not apparent until 5 days after immunization with S-III. They attributed the additional PFC to additional rounds of proliferation of PFC (64). To determine when the amplification induced by Con A was first obvious, mice were immunized with 0.25 μ g PVP and treated with 150 μ g of Con A 2 days later. PVP-specific PFC were determined on days 3, 4, or 5 after

TABLE 2. EFFECT OF CON A ON THE MAGNITUDE OF THE PVP-SPECIFIC PFC RESPONSE IN NUDE AND NORMAL BALB/C MICE.

MICE	CON A ^a	PVP-SPECIFIC PFC ^b	
		SPLEEN	10 ⁶
NUDE	NONE	8,900 ± 1,073	66 ± 16
NUDE	DAY 0	5,825 ± 2,903	38 ± 23
NUDE	DAY +2	9,205 ± 8,878	60 ± 60
NORMAL	NONE	11,150 ± 6,425	73 ± 29
NORMAL	DAY 0	1,325 ± 366	7 ± 0.82
NORMAL	DAY +2	90,375 ± 34,574	323 ± 160

^aCON A (150 µg) WAS GIVEN I.V. EITHER ON DAY 0 OR DAY +2 WITH RESPECT TO IMMUNIZATION WITH PVP.

^bARITHMETIC MEAN ± S.D. FOR 5 MICE, DETERMINED 5 DAYS AFTER IMMUNIZATION WITH 0.25 µg PVP.

immunization. The results (Fig. 2) show that the effects of Con A treatment on PFC generation were evident by day 5. Thus, Con A treatment did not affect the number of PFC generated before day 4 and required 2-3 days after Con A treatment to reach maximal proportions.

Kinetics of the Con A-induced suppression of the PVP-specific PFC response

Kinetic studies by Rich and Pierce of Con A induced suppression in vitro showed that the suppression occurred during the log phase of PFC production to SRBC, about 4 days after addition of Con A and initiation of cultures (51). Markham et al. (63) observed similar results in vivo when monitoring Con A induced suppression to S-III. Fig. 3 shows that Con A induced suppression of PVP-specific PFC was already evident by day 4 of the response. The number of PFC declined further on day 5 when the difference was maximal between Con A treated and control mice.

Effect of Con A on established low dose paralysis to PVP

Baker et al. (49) have presented evidence that low dose paralysis to S-III may be mediated by suppressor T cells. Previous evidence indicated that low dose paralysis to PVP also may be mediated by suppressor cells (48). Recent work by Markham et al. (64) demonstrated that Con A, given at a time for its amplifying effect, partially

Figure 2. Kinetics of Con A-induced amplification of the PVP-specific PFC response. Con A-treated mice (Δ — Δ) received 150 μ g i.v. 2 days after immunization with 0.25 μ g PVP. Control mice received PVP only (o—o). Each point represents the mean PFC determined in 5 mice.

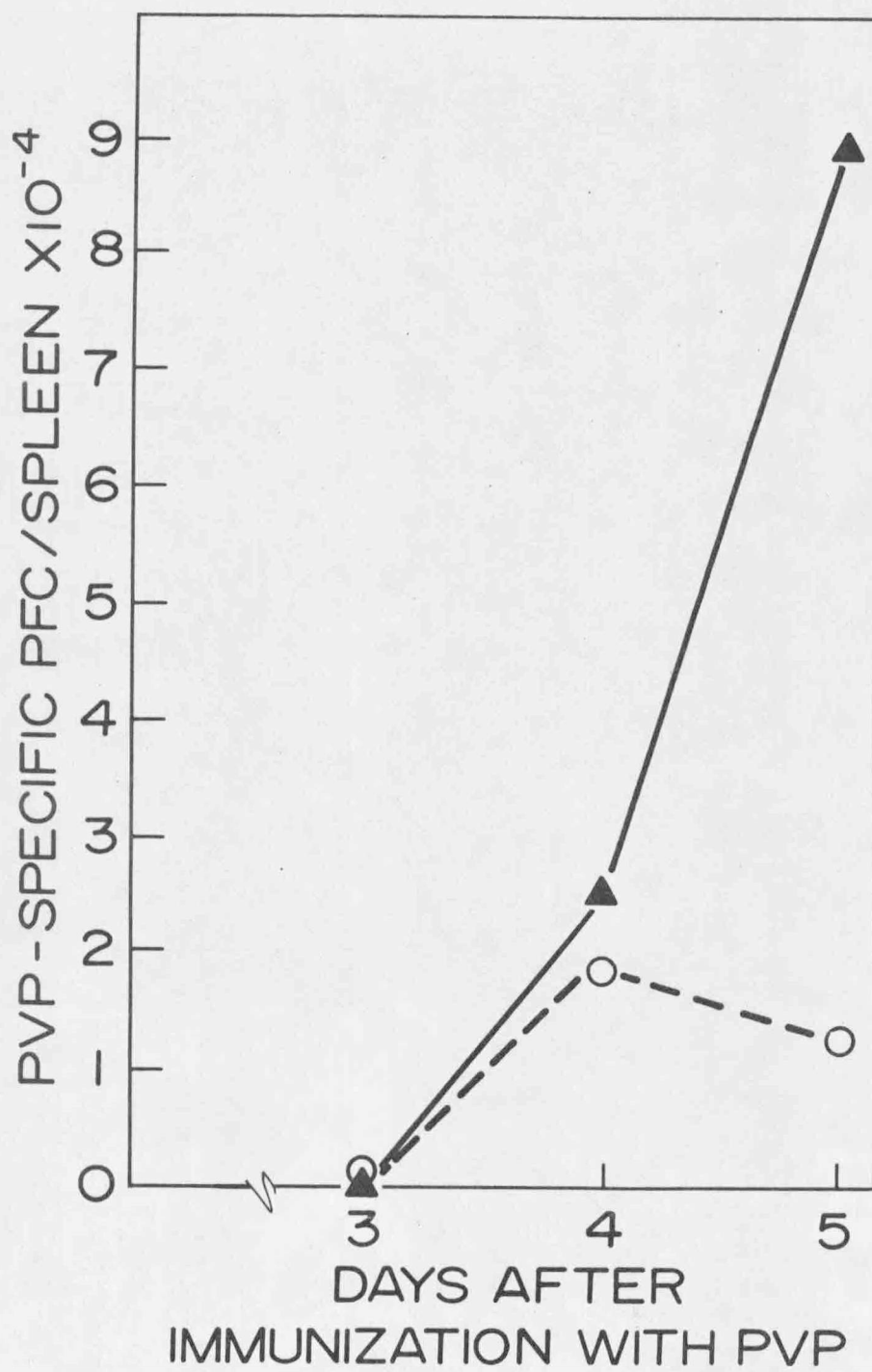
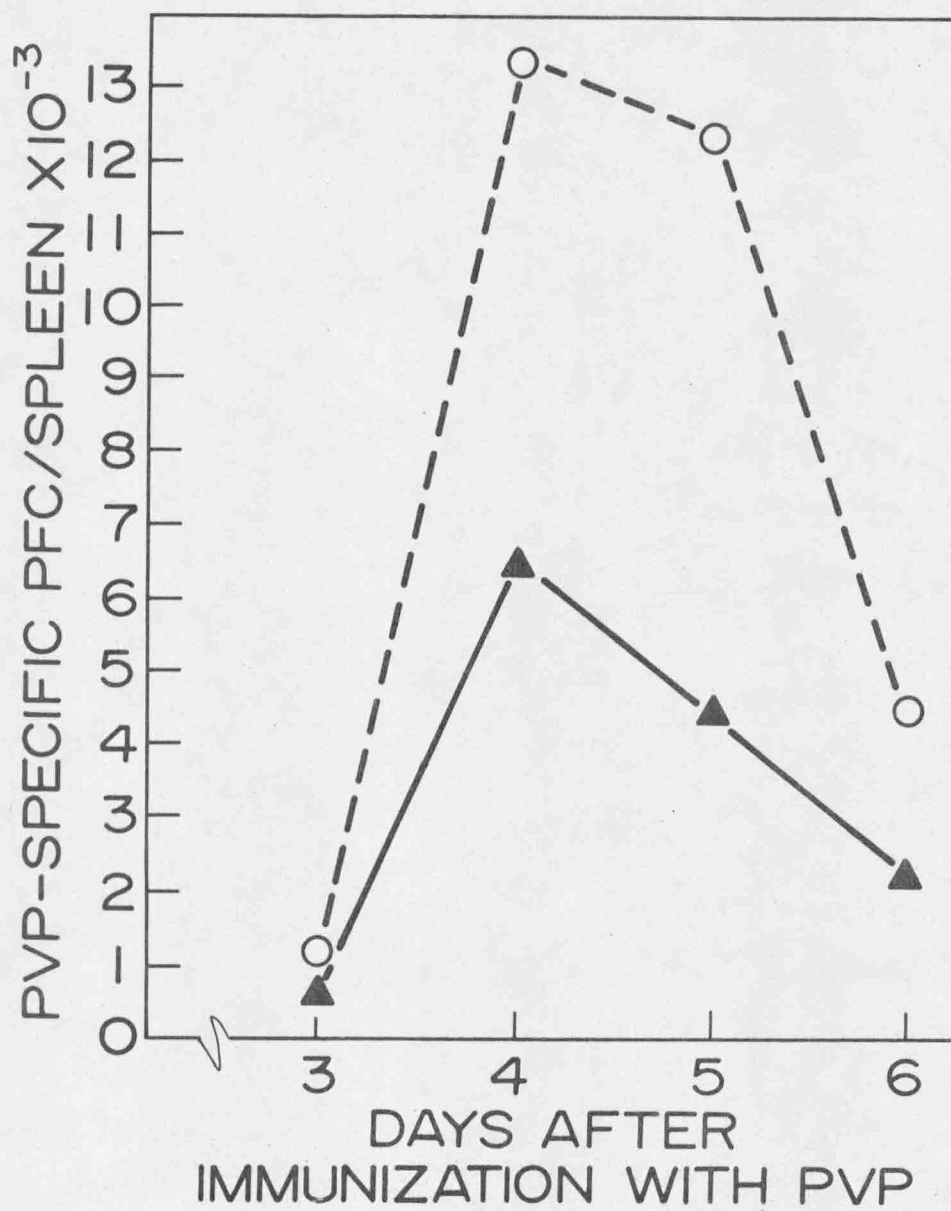


Figure 3. Kinetics of Con A-induced suppression of the PVP-specific PFC response. Con A-treated mice (▲—▲) received 150 μ g i.v. on the day of immunization with PVP (0.25 μ g). Control mice (○—○) received PVP only. Each point represents the mean PFC of 5 mice.



reversed the low dose paralytic state of S-III. Because the immunoregulatory mechanisms of S-III and PVP appear to be similar in many aspects, it was of interest to determine the effect of Con A on a state of low dose paralysis to PVP.

Low doses paralysis to PVP was induced by injecting mice with 0.025 μ g of PVP 3 days before challenge with 0.25 μ g PVP. Con A (150 μ g) was given 2 days after challenge with PVP. The results (Table 3) show that Con A treatment of low dose paralyzed mice almost completely overcame the effect of low dose paralysis (Group C vs Group D).

Effect of ALS on Con A-induced suppression of the PVP-specific PFC response

Con A-induced suppression may be due to activation of suppressor T cells (Table 2). Lake and Reed previously showed that ALS treatment increased the magnitude of the PFC response to PVP which they attributed to possible removal of suppressor T cells by the ALS treatment (98). Therefore, if Con A was activating suppressor T cells, it may be possible to remove them by ALS treatment.

To test this possibility, mice were given 150 μ g of Con A 1 day before immunization with PVP. ALS (0.3 ml) was given immediately after immunization with PVP. Table 4 shows that ALS-treatment of Con A suppressed mice caused a 4-fold increase in PFC when compared with Con A suppressed mice (Group C vs. Group B). This increase in PFC may be

TABLE 3. EFFECT OF CON A ON ESTABLISHED LOW DOSE PARALYSIS TO PVP.

GROUP	TREATMENT		PVP-SPECIFIC PFC ^c	
	PRIME ^a	CON A ^b	SPLEEN	10 ⁶
A	-	+	12,038 ± 4,926	93 ± 34
B	+	-	2,650 ± 795	21 ± 5
C	+	+	117,208 ± 22,300	359 ± 56
D	-	+	144,613 ± 62,955	429 ± 128

^aLOW DOSE PARALYSIS WAS INDUCED BY PRIMING ANIMALS WITH 0.025 µg PVP I.V. 3 DAYS BEFORE CHALLENGE WITH 0.25 µg PVP.

^bCON A (150 µg) WAS GIVEN I.V. 2 DAYS AFTER CHALLENGE WITH 0.25 µg PVP.

^cARITHMETIC MEAN ± S.D. FOR 6 ANIMALS, DETERMINED 5 DAYS AFTER IMMUNIZATION WITH PVP.

TABLE 4. EFFECT OF ALS TREATMENT ON CON A-INDUCED SUPPRESSION OF THE PVP-SPECIFIC PFC RESPONSE.

GROUP	TREATMENT		PVP-SPECIFIC PFC/SPL ^c
	CON A ^a	ALS ^b	
A	-	-	15,755 ± 7,196
B	+	-	7,035 ± 2,860
C	+	+	31,371 ± 10,187
D	-	+	193,458 ± 74,551

^aCON A (150 µg) WAS GIVEN I.V. 24 HOURS BEFORE IMMUNIZATION WITH 0.25 µg PVP.

^bALS (0.3 ML) WAS GIVEN I.V. IMMEDIATELY FOLLOWING IMMUNIZATION.

^cARITHMETIC MEAN ± S.D. FOR 5-6 MICE, DETERMINED 5 DAYS AFTER IMMUNIZATION WITH 0.25 µg PVP.

attributed to removal of Con A-activated suppressor cells by the ALS. Similar findings have been reported by Markham *et al.* (63) using S-III.

Effect of different doses of silica on the PVP-specific PFC response

The requirement for macrophages in antibody responses to TI antigens has been an area of controversy (9-11). Silica, a specific macrophage toxin, was given *in vivo* in an attempt to delineate the role of macrophages in TI antibody responses. However, preliminary experiments did not show the expected decrease in antibody response if the macrophages had a significant role in antibody responses to TI antigens, but an unexpected increase in antibody response to PVP, a TI antigen. Thus the following experiments were designed to attempt to find the mechanism of silica induced amplification of the PFC response to PVP.

Table 1 showed that different doses of Con A amplified the PFC responses to PVP to different levels. Therefore, it was important to determine the effect of different doses of silica on the PFC response to PVP.

In an initial experiment, silica was suspended in PBS and injected i.v. in doses of 1, 2.5, 5, and 10 mg in 0.5 ml 2 days after immunization with the optimal immunogenic dose of PVP (0.25 μ g). The PFC values were determined 5 days after immunization. The data of Table 5 show that 2.5 mg was as effective in amplifying the PFC responses as 5 mg. Mice receiving 10 mg of silica all died within 24 hours of the

TABLE 5. EFFECT OF DIFFERENT DOSES OF SILICA ON THE PVP-SPECIFIC PFC RESPONSE.

DOSE OF SILICA ^a	PVP-SPECIFIC ^b PFC/SPLEEN
0	10,530 $\pm$ 3,218
1 MG	21,600 $\pm$ 5,768
2.5 MG	51,455 $\pm$ 10,078
5 MG	52,110 $\pm$ 12,691

^aTHE VARIOUS DOSES OF SILICA WERE GIVEN I.V. 2 DAYS AFTER IMMUNIZATION WITH PVP.

^bARITHMETIC MEAN $\pm$ S.D. FOR 5-6 MICE, DETERMINED 5 DAYS AFTER IMMUNIZATION WITH 0.25 μ g PVP.

silica injection (data not shown). Mice were subsequently given 2.5 mg of silica to induce amplification of the PVP specific PFC response.

Effect of silica on the magnitude of the PVP-specific PFC response given at different times with respect to immunization

Pearsall and Weiser observed that silica given at various times with respect to skin grafting altered the rejection times of skin allografts (75). The timing of Con A administration was also shown to effect the PVP-specific PFC response (Figure 1). Clearly, the time of silica treatment with respect to immunization needed to be evaluated.

To observe the effect of differently timed silica injections, a single dose of silica (2.5 mg) was given i.v. to mice on days -3, -2, -1, 0, +1, +2, +3, and +4 with respect to immunization. All mice received 0.25 μ g PVP on day 0 and their PFC responses were determined on day 5. Figure 4 shows that mice injected with silica on days -2 to -1 or +2 demonstrated the greatest increase in PFC responses to PVP.

Effect of multiple injections of silica on the PVP-specific PFC response

It was of interest to see if further amplification was possible if multiple injections were given. Mice were given 2.5 mg silica on days -2 and +2 with respect to immunization. Interestingly, mice given a single injection of silica did not respond as well as mice given silica on both day -2 and +2 (Table 6). This increase in the PFC response of mice receiving 2 injections of silica was not due to splenomegaly

Figure 4. Magnitude of the PVP-specific PFC response in mice given silica (2.5 mg) at different times relative to immunization with 0.25 μ g PVP. Each point represents the mean PFC of 5 mice determined 5 days after immunization with PVP.

MAGNITUDE OF THE PFC RESPONSE TO PVP
IN MICE GIVEN SILICA AT DIFFERENT TIMES
RELATIVE TO IMMUNIZATION

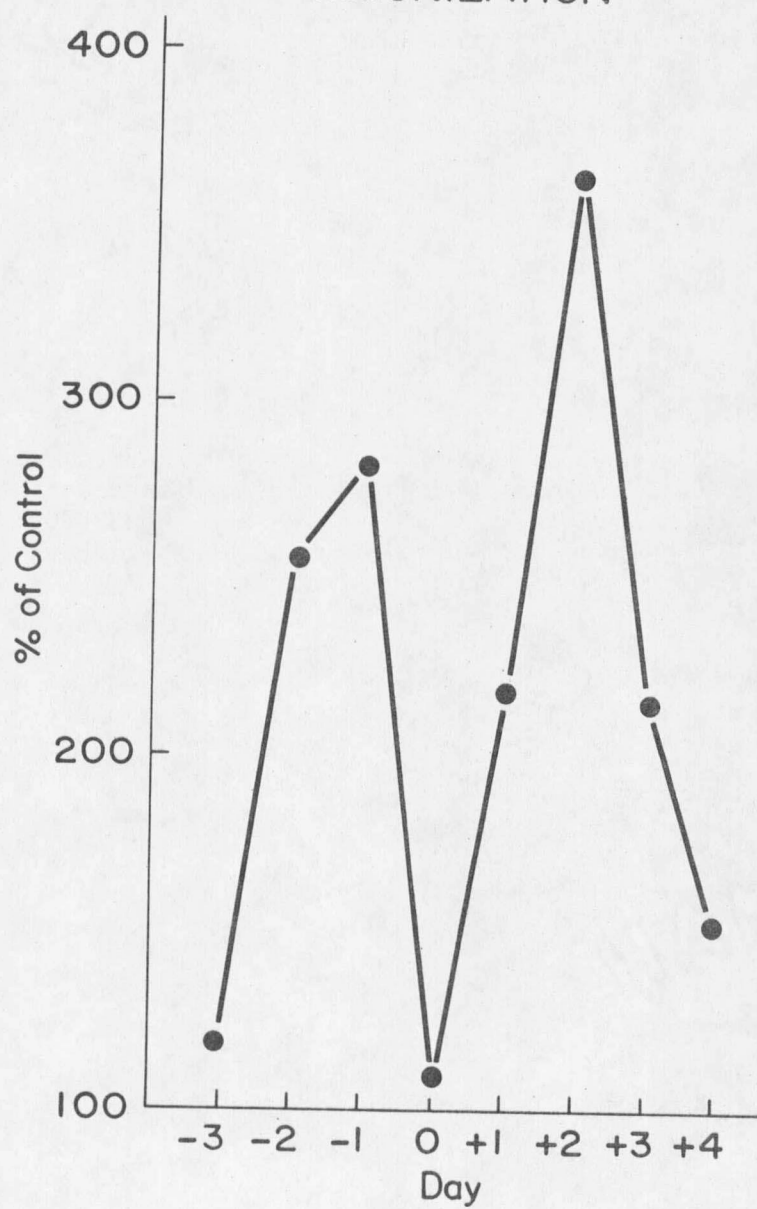


TABLE 6. EFFECT OF ONE OR TWO INJECTIONS OF SILICA ON THE MAGNITUDE OF THE PVP-SPECIFIC PFC RESPONSE.

GROUP	SILICA ^a		PVP-SPECIFIC PFC ^b	
	DAY -2	DAY +2	SPLÉEN	10 ⁶
1	-	-	12,450 ± 5,335	109 ± 30
2	+	-	75,260 ± 11,887	223 ± 61
3	-	+	70,595 ± 36,088	215 ± 43
4	+	+	181,600 ± 20,337	572 ± 94

^aSILICA (2.5 MG) IN PBS WAS GIVEN I.V. EITHER 2 DAYS BEFORE OR 2 DAYS AFTER IMMUNIZATION WITH PVP.

^bARITHMETIC MEAN ± S.D. FOR 5 MICE DETERMINED 5 DAYS AFTER IMMUNIZATION WITH 0.25 µg PVP.

alone because the number of PFC/ 10^6 spleen cells was about 5 times greater in this group when compared with control mice not receiving silica.

Although it was apparent silica could amplify PFC responses to PVP, the mechanism of silica-induced amplification was not known. The following experiments were designed to elucidate the possible mechanism (s) of amplification.

Effect of route of administration of silica on the PVP-specific PFC response

Lake previously determined that amplification of the PFC response to PVP occurred only if the ALS and PVP were administered via the same route (J.P. Lake, personal communication). In light of this fact, it was important to determine if the route of administration of silica influenced its effect on the immune response to PVP. Groups of mice were given either 2.5 or 10 mg of silica i.p. on days -2 and +2 and were compared with a group given 2.5 mg of silica i.v. by the same injection schedule. The results (Table 7) show that either amount of silica administered i.p. failed to increase the PFC response to PVP when compared with normal mice and mice receiving silica i.v. These data indicate that the route of silica administration was important for amplification.

TABLE 7. EFFECT OF ROUTE OF ADMINISTRATION OF SILICA ON THE PVP-SPECIFIC PFC RESPONSE.

GROUP	SILICA TREATMENT ^a		PVP-SPECIFIC PFC/SPL ^b
	I.P.	I.V.	
A	-	-	16,885 $\pm$ 5,294
B	2.5 MG	-	21,070 $\pm$ 16,702
C	10.0 MG	-	27,290 $\pm$ 16,393
D	-	2.5 MG	128,885 $\pm$ 79,526

^aSILICA WAS ADMINISTERED BY THE INDICATED ROUTE 2 DAYS BEFORE AND 2 DAYS AFTER IMMUNIZATION WITH PVP.

^bARITHMETIC MEAN $\pm$ S.D. FOR 5 MICE, DETERMINED 5 DAYS AFTER IMMUNIZATION WITH 0.25 μ g PVP.

Kinetics of the silica-induced amplified PVP-specific PFC response

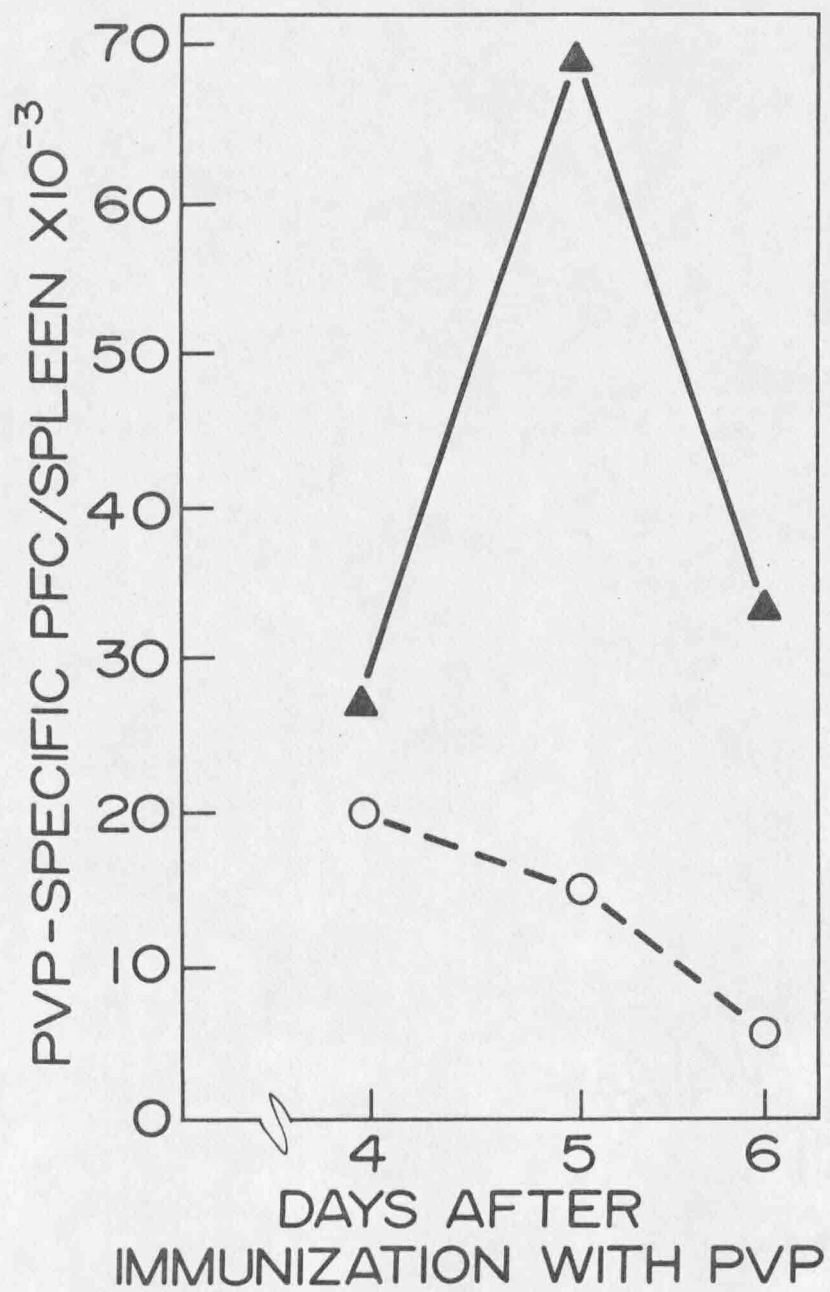
Kinetic studies of the Con A-induced amplification of the PFC response to PVP (Figure 2) showed that amplification was not apparent until day 5. A similar study of the kinetics of the silica induced amplification hopefully would lead to some insight into the mechanism of the amplification.

To study the kinetics of the amplification, silica (2.5 mg) was given to mice on days -2 and +2 with respect to immunization with PVP. The PFC values were determined on days 4, 5, and 6 after immunization and compared with the PFC values of mice not given silica. Figure 5 shows that both silica-treated and control mice generated about the same number of PVP-specific PFC on day 4. However, on day 5 the silica-treated mice showed a significant increase in the number of PFC when compared with normal mice ($p < 0.05$). The number of PFC declined in both silica-treated and control mice on day 6. The kinetics of the silica-induced amplification closely paralleled the kinetics of the Con A-induced amplification of the PFC response. This similarity may possibly indicate a similar mechanism of amplification.

Effect of silica on the serum antibody titer to PVP

Baker et al. (107) noted that ALS treatment of mice increased the number of S-III specific PFC 10-fold, but, paradoxically, the hemolytic serum titer was increased only slightly. Thus, it was of interest to

Figure 5. Kinetics of the silica-induced amplification of the PVP-specific PFC response. Silica (2.5 mg) was given i.v. 2 days before and 2 days after immunization with 0.25 μ g PVP. Each point represents the mean number of PFC determined from 5 mice at various times after immunization with PVP. Silica treated mice are denoted as (▲—▲) and control mice as (○—○).



examine the effect of silica on the serum antibody titer to PVP.

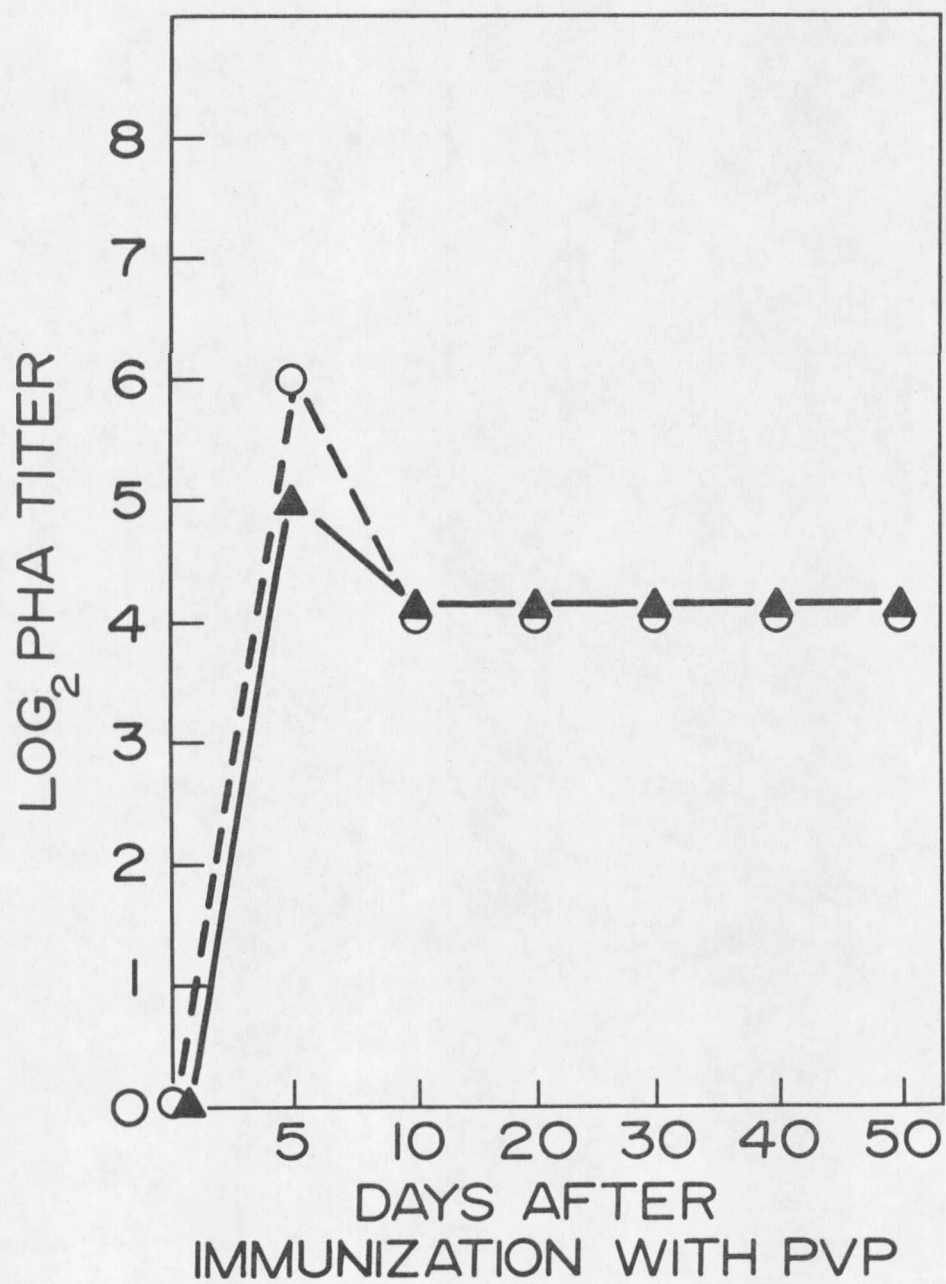
The serum antibody titer of normal and silica-treated mice was determined by use of a microtiter assay on various days following immunization with PVP. Mice were given silica on days -2 and +2 with respect to immunization. Control mice were given PBS by the same injection schedule as silica-treated mice. The results (Figure 6) show that both silica-treated and control mice had a peak serum antibody titer on day 5. Although the silica-treated mice had a slightly higher titer than control mice, the difference was not significant. By day 10 there was no difference between the two groups.

Comparison of the effects of a reference silica and Min-U-Sil on the PVP-specific PFC response

Robock has proposed the use of a reference silica (DQ12<5 μ m) in research concerning the effects of silica in animal models (103). Therefore, it was important to compare the effects of Min-U-Sil used in the previous experiments with those produced by DQ12.

DQ12 was prepared in the same manner as the Min-U-Sil fraction III except that the fractionation step was excluded. Mice were treated with DQ12 of Min-U-Sil as previously described i.e. 2.5 mg were given i.v. 48 hours before and after immunization with PVP. Both the DQ12 and the fraction III Min-U-Sil produced similar degrees of amplification of the PVP-specific PFC response indicating they were comparable in

Figure 6. Effect of silica on the serum antibody response to PVP. Balb/c mice were given 2.5 mg silica 2 days before and 2 days after immunization with 0.25 μ g PVP. Serum was pooled and titered without further treatment. Each point represents 7 mice. Silica treated mice are denoted as (○—○) and untreated control mice as (▲—▲).



their effects (Table 8). This observation also tended to discount any unique effect the Min-U-Sil may have had on the immune response to PVP.

Effect of silica on the phagocytic ability of mice

The carbon clearance assay is often used to measure the phagocytic ability of mice (105). Friedman and Moon (70) indicated that DQ12 was effective in depressing phagocytic ability but Min-U-Sil was ineffective. Therefore, it was important to confirm these observations. Mice were given 2.5 mg of either fraction III Min-U-Sil or DQ12 i.v. 18-20 hours before their phagocytic ability was determined by the carbon clearance assay of Biozzi et al. (105). Table 9 shows that silica-treated mice were depressed in their ability to clear carbon from their blood when compared with normal mice. The results also indicated that the degree of depression caused by Min-U-Sil and DQ12 was similar.

Effect of silica on the magnitude of the PVP-specific PFC response in nude and normal mice

Lake and Reed previously demonstrated that the PFC response to PVP may be regulated by a subpopulation of T cells (6). The amplification produced by silica treatment may have been due to (among many possibilities) (i) removal or inactivation of suppressor cells or their activity or (ii) removal or inactivation of a macrophage population which regulated PVP-specific PFC responses.

TABLE 8. COMPARISON OF THE EFFECTS OF A REFERENCE SILICA AND MIN-U-SIL ON THE PVP-SPECIFIC PFC RESPONSE.

GROUP	SILICA ^a		PVP-SPECIFIC PFC ^b	
	DQ12	FRAC III	SPLEEN	10 ⁶
A	-	-	10,145 ± 1,694	112 ± 17
B	+	-	125,665 ± 42,578	362 ± 86
C	-	+	141,860 ± 36,138	337 ± 76

^aDQ12 OR FRACTION III (2.5 MG) WAS GIVEN I.V. 2 DAYS BEFORE AND 2 DAYS AFTER IMMUNIZATION WITH PVP.

^bARITHMETIC MEAN ± S.D. FOR 5 MICE, DETERMINED 5 DAYS AFTER IMMUNIZATION WITH 0.25 µg PVP.

TABLE 9. EFFECT OF SILICA ON THE PHAGOCYtic ABILITY OF MICE.

TYPE OF SILICA ^a	PHAGOCYtic INDEX ^b	P
CONTROL	0.117 ± 0.006	-
DQ12	0.090 ± 0.008	P<0.05
MIN-U-SIL	0.085 ± 0.008	P<0.05

^aSILICA (2.5 MG) WAS GIVEN I.V. 18-20 HOURS BEFORE THE PHAGOCYtic ABILITY OF THE MICE WAS DETERMINED.

^bAVERAGE VALUE ± S.D. OF 4-5 MICE.

To aid in distinguishing between these latter two possibilities, groups of nude and their NLM were injected with 2.5 mg of silica i.v. on days -2 and +2 with respect to immunization. All mice received the optimal immunogenic dose of PVP on day 0 and their PFC responses were determined on day 5. Table 10 shows that NLM treated with silica showed about a 6-fold increase in PVP-specific PFC. This increase was statistically significant ($p < 0.005$). In contrast, silica treated nude mice showed only a 2-fold increase in PFC value which was not statistically significant ($p > 0.05$). These results suggest that T cells are required for the marked amplification in NLM.

Effect of silica on Con A-induced suppression to PVP

Previous experiments (Fig. 1 and Table 2) established that Con A treatment of mice at the time of antigen challenge suppressed the PFC response to PVP and that the suppression was thymus dependent. The suppression resulting from Con A treatment may have been due to a non-specific activation of suppressor cells. The amplification induced by silica of the PFC response to PVP may have been due to nonspecific inactivation or alteration of function of suppressor cells which have been proposed to govern the immune response to PVP. If the latter possibility was true, it may be possible to relieve some of the Con A-induced suppression by silica treating Con A suppressed mice.

TABLE 10. EFFECT OF SILICA ON THE MAGNITUDE OF THE PVP-SPECIFIC PFC RESPONSE IN NUDE AND NORMAL BALB/C MICE.

GROUP	NO. OF ANIMALS	SILICA ^a	PVP-SPECIFIC PFC/SPL ^b
NUDE	8	-	10,544 ± 6,129
NORMAL	8	-	13,675 ± 8,131
NUDE	8	+	21,197 ± 7,882
NORMAL	8	+	87,147 ± 31,819

^aSILICA (2.5 MG) WAS GIVEN I.V. ON DAYS -2 AND +2 WITH RESPECT TO IMMUNIZATION WITH PVP.

^bARITHMETIC MEAN ± S.D. FOR 8 MICE, DETERMINED 5 DAYS AFTER IMMUNIZATION WITH 0.25 µg PVP.

To test this hypothesis, mice were treated with Con A, silica, or with a combination of silica and Con A. Con A (150 μ g) was given at the time of immunization with PVP to suppress the immune response to PVP. Silica (2.6 mg) was given 2 days before and 2 days after immunization with PVP. The results (Table 11) show that Con A suppressed the PFC response to PVP as in previous experiments (Table 2). Silica treated mice had an amplified PFC response to PVP, also in agreement with previous experiments (Table 10). However, mice treated with both Con A and silica responded at a level comparable with that of control mice (Group C vs. Group A). The increase was shown in both PFC/spleen and PFC/ 10^6 . These findings suggest that silica treatment was able to partially reverse the suppressing effects of Con A perhaps by affecting the activity of suppressor cells.

Effect of silica on the PFC response to SRBC

Pernis and Paronetto reported that i.v. administered silica produced a more than 10-fold increase in antibody titer of rabbits immunized with ovalbumin (86). Because the types of silica used in their studies differed from the type of silica used in this study, it was of interest to determine if the fraction III used would increase the PFC response to a TD antigen such as SRBC.

Silica (2.5 mg) was given 2 days before and 2 days after immunization with 0.25 ml of a 10% suspension of SRBC. Table 12 shows that

TABLE 11. EFFECT OF SILICA ON CON A-INDUCED SUPPRESSION OF MICE OF THE IMMUNE RESPONSE TO PVP.

GROUP	TREATMENT		PVP-SPECIFIC PFC ^c	
	CON A ^a	SILICA ^b	SPLEEN	10 ⁶
A	-	-	21,875 ± 13,437	156 ± 82
B	+	-	2,695 ± 493	14 ± 7
C	+	+	21,875 ± 9,103	60 ± 24
D	-	+	91,640 ± 26,508	446 ± 188

^aCON A (150 µg) WAS GIVEN I.V. AT THE SAME TIME AS IMMUNIZATION WITH PVP.

^bSILICA (2.5 MG) WAS GIVEN I.V. 2 DAYS BEFORE AND 2 DAYS AFTER IMMUNIZATION WITH PVP.

^cARITHMETIC MEAN ± S.D. FOR 5 MICE, DETERMINED 5 DAYS AFTER IMMUNIZATION WITH 0.25 µg PVP.

TABLE 12. EFFECT OF SILICA ON THE PFC RESPONSE OF MICE TO SRBC.

GROUP	SILICA ^a	PFC/SPLEEN ^b
A	+	115,625 ± 17,700
B	-	64,219 ± 8,947

^aSILICA (2.5 MG) WAS GIVEN I.V. 2 DAYS BEFORE AND 2 DAYS AFTER IMMUNIZATION WITH SRBC.

^bARITHMETIC MEAN ± S.D. FOR 4 MICE, DETERMINED 5 DAYS AFTER IMMUNIZATION WITH 0.25 ML OF A 10% SUSPENSION OF SRBC.

silica treated mice had about a 2-fold increase in PFC/spleen to SRBC. However, this increase in PFC was not statistically significant ($p > 0.05$).

Effect of silica on the PFC responses to LPS and S-III

The antigens LPS, S-III, and PVP have all been shown to be TI (5,6). Because silica treatment increased responses to one TI antigen PVP, it was of interest to determine if silica treatment would also increase PFC responses to other TI antigens.

Mice were treated with silica as previously described for enhancement of PFC responses to PVP (Table 10) and immunized with either 10 μ g of E. coli 0113 LPS or 0.5 μ g of S-III. Experiments involving silica and S-III were done by Dr. Phillip J. Baker, NIH, Bethesda, MD.

Silica treatment of mice immunized with LPS showed a 2-fold amplification of the PFC response (Table 13) which was also reflected in the PFC/ 10^6 . Table 14 shows that silica treatment was capable of modest amplification of PFC responses to S-III (expt. #1) but did not amplify the responses in a subsequent experiment (expt. #2). The failure to amplify the immune response to LPS was not surprising because there has been no evidence for suppressor T cell regulation of the immune response to this antigen. However, the lack of effect of silica treatment on the immune response to S-III was surprising because many aspects of the immune response e.g. regulatory mechanisms,

TABLE 13. EFFECT OF SILICA ON THE LPS-SPECIFIC PFC RESPONSE OF MICE.

GROUP	SILICA ^a	LPS-SPECIFIC PFC ^b	
		SPLEEN	10 ⁶
A	-	4,813 ± 2,198	38 ± 12
B	+	10,056 ± 4,358	77 ± 30

^aSILICA (2.5 MG) WAS GIVEN I.V. 2 DAYS AFTER IMMUNIZATION WITH LPS.

^bARITHMETIC MEAN ± FOR 4 MICE, DETERMINED 4 DAYS AFTER IMMUNIZATION WITH 10 µg LPS I.V.

TABLE 14. EFFECT OF SILICA ON THE S-III SPECIFIC PFC RESPONSE.

GROUP	SILICA ^a	S-III-SPECIFIC PFC ^b	
		EXPT #1	EXPT #2
A	-	4.359 ± 0.112 (22,842)	4.203 ± 0.048 (15,965)
B	+	4.885 ± 0.048 (76,671)	4.143 ± 0.129 (13,905)

^aSILICA (2.5 MG) WAS GIVEN I.V. 2 DAYS BEFORE AND 2 DAYS AFTER IMMUNIZATION WITH S-III.

^bLOG₁₀ MEAN ± S.D. FOR 9-10 MICE, DETERMINED 5 DAYS AFTER IMMUNIZATION WITH 0.5 µg S-III; GEOMETRIC MEANS ARE IN PARENTHESES.

to both S-III and PVP were similar. The lack of amplification of S-III responses may be an indication of a more fundamental difference in the immune response to S-III and PVP.

Effect of silica and Con A on the PVP-specific PFC response to PVP

Con A had previously been shown to amplify the PFC response to PVP if given at the proper time with respect to immunization (Figure 1). The mechanism of amplification in Con A-treated mice may be different than the mechanism of amplification induced by silica treatment. Both share a common requirement in that T cells are needed for amplification (Table 2 and Table 10). Therefore, if the mechanisms were different, mice treated with both Con A and silica might show additive amplification.

To test this possibility, mice were given 150 μ g of Con A days after immunization with PVP and also 2.5 mg of silica on days -2 and +2 with respect to immunization. Table 15 shows that the group of mice receiving both silica and Con A (Group D) did not show additive amplification of the PVP-specific PFC response which would have been expected if the treatments were exerting their effects via different mechanisms. These results suggest that both treatments may be affecting the response to PVP by the same mechanism.

TABLE 15. EFFECT OF SILICA AND CON A ON THE PVP-SPECIFIC PFC RESPONSE.

GROUP	TREATMENT		PVP-SPECIFIC PFC ^c	
	CON A ^a	SILICA ^b	SPLEEN	10 ⁶
A	-	-	12,913 ± 3,430	125 ± 29
B	-	+	78,865 ± 32,310	380 ± 150
C	+	-	130,030 ± 56,338	472 ± 161
D	+	+	111,605 ± 29,603	379 ± 177

^aCON A (150 µg) WAS GIVEN I.V. 2 DAYS AFTER IMMUNIZATION WITH PVP.

^bSILICA (2.5 MG) WAS GIVEN I.V. 2 DAYS BEFORE AND 2 DAYS AFTER IMMUNIZATION WITH PVP.

^cARITHMETIC MEAN ± S.D. FOR 4-5 MICE, DETERMINED 5 DAYS AFTER IMMUNIZATION WITH 0.25 µg PVP.

Effect of silica on low dose paralysis to PVP

Some adjuvants, such as LPS, can prevent or abrogate tolerance to an antigen when administered in conjunction with that antigen (108). Other studies have shown that silica may have some adjuvant-like properties (86-88). Because silica may have some adjuvant-like properties, it was of interest to determine its effect on a state of tolerance to PVP.

Low dose paralysis to PVP was induced by priming mice with 0.025 µg PVP as previously described (47), 5 days before challenge with the optimal immunogenic dose of PVP i.e. day -5. On days -2 (3 days after priming for low dose paralysis) and +2 (2 days after challenge with the optimal immunogenic dose of PVP) mice were given 2.5 mg of silica i.v.

The results (Table 16) show that low dose paralysis was induced in primed mice when compared with mice that were challenged only (Group A vs. Group B). The mice of Group D show the marked amplification of the PFC response by silica treatment. The primed, silica treated, and challenged mice (Group C) had PFC responses which were nearly identical to the responses of normal untreated mice (Group C vs. Group A) indicating that the tolerant state was partially reversed by silica treatment. Because low dose paralysis to PVP may be mediated by suppressor cells (48), the increase again raises the possibility

TABLE 16. EFFECT OF SILICA ON LOW DOSE PARALYSIS TO PVP.

GROUP	TREATMENT		PVP-SPECIFIC PFC ^c	
	PRIME ^a	SILICA ^b	SPLEEN	10 ⁶
A	-	-	24,870 ± 5,725	178 ± 33
B	+	-	4,360 ± 1,103	34 ± 6
C	+	+	24,535 ± 4,741	70 ± 8
D	-	+	256,605 ± 54,886	532 ± 90

^aLOW DOSE PARALYSIS WAS INDUCED BY PRIMING MICE WITH 0.025 μ g PVP 3 DAYS BEFORE SILICA TREATMENT AND 5 DAYS BEFORE IMMUNIZATION.

^bSILICA (2.5 MG) WAS GIVEN I.V. 3 DAYS AFTER PRIMING WITH 0.025 μ g PVP AND 2 DAYS BEFORE AND 2 DAYS AFTER IMMUNIZATION.

^cARITHMETIC MEAN ± S.D. FOR 5 MICE, DETERMINED 5 DAYS AFTER IMMUNIZATION WITH 0.25 μ g PVP.

that silica may be interfering with the activity of suppressor cells in some manner.

Effect of PVNO on silica-induced amplification to PVP

Previous reports demonstrated that administration of poly-2-vinylpyridine N-oxide (PVNO), a macrophage stabilizing agent, would prevent the deleterious effects of silica on macrophages (76). By protecting macrophages against the effects of silica, it was hoped to gain a clearer understanding into the effects of silica on the immune response to PVP.

Mice were given 4 mg of PVNO subcutaneously 24 hours before each silica injection. Silica (2.5 mg) was given 2 days before and 2 days after immunization with PVP. Interestingly, Table 17 shows that both group B and the silica treated group C that received the PVNO were essentially unresponsive. This unresponsiveness to PVP may be due to (i) an untoward effect by PVNO on the cells involved in the immune response or (ii) a state of immunologic unresponsiveness to PVP since PVNO is structurally similar to PVP. This result was interesting because tolerance classically was considered to be antigen specific. Although tolerance may be broken by challenge with cross-reacting antigens, the results here may represent tolerance induction by a structural analog.

TABLE 17. EFFECT OF PVNO ON SILICA-INDUCED ENHANCEMENT OF THE PVP-SPECIFIC PFC RESPONSE.

GROUP	TREATMENT		PVP-SPECIFIC PFC ^c	
	PVNO ^a	SILICA ^b	SPLEEN	10 ⁶
A	-	-	12,695 ± 3,725	112 ± 25
B	+	-	1,115 ± 1,183	13 ± 13
C	+	+	2,863 ± 1,697	23 ± 12
D	-	+	76,020 ± 40,913	413 ± 163

^aPVNO (4 MG) WAS GIVEN S.C. 24 HOURS BEFORE EACH SILICA INJECTION I.E. ON DAYS -3 AND +1 WITH RESPECT TO IMMUNIZATION WITH 0.25 µg PVP.

^bSILICA (2.5 MG) WAS GIVEN I.V. ON DAYS -2 AND +2 WITH RESPECT TO IMMUNIZATION.

^cARITHMETIC MEAN ± S.D. FOR 5-6 MICE, DETERMINED 5 DAYS AFTER IMMUNIZATION WITH PVP.

DISCUSSION

Previous reports have established that Con A added at the time of initiation of spleen cell cultures suppressed antibody responses to both TD (51) and TI (52) antigens. Con A could amplify antibody responses if added at the appropriate time, usually 24-48 hours after culture initiation, to both TD (109) and TI (110) antigens. This suppression and amplification were thymus dependent; spleen cells from nude mice or thymectomized, irradiated, bone marrow reconstituted mice did not suppress or amplify antibody responses after culture with Con A (53,110). The few comparable studies done using intact animals and TI antigens prompted the present study. The use of normal and congenitally thymus-deficient mice offered a simple and reliable system to study the effects of Con A on immune responses to PVP.

The data of Figure 1 show that the time of Con A administration determined whether the response to PVP would be suppressed or amplified. Con A given 1-2 days before immunization or at the time of immunization suppressed PFC responses to PVP. In contrast, Con A given 2 days after immunization resulted in a 5-10 fold amplification of the PVP-specific PFC response.

Because the immune response to PVP may be regulated by suppressor cells (6), it was important to determine if the Con A-induced suppression or amplification was T cell dependent. Table 2 shows that Con A-treatment of nude mice resulted in neither suppression nor amplification

of the PVP-specific PFC response indicating that both suppression and amplification were T cell dependent.

Baker has proposed that two thymus-dependent cells regulate the immune response to S-III, a TI antigen (42). ALS-treatment of normal mice produces a 5-10 fold amplification of the S-III specific PFC response. Infusion of syngeneic thymocytes into ALS-treated mice abrogated the ALS-induced amplification. In contrast, infusion of peripheral blood lymphocytes into ALS-treated mice produced additional amplification. On the basis of these observations, Baker proposed that two thymus-dependent cells, termed suppressor and amplifier cells, act in an opposing manner to regulate immune responses to S-III (42). Baker has obtained additional evidence which indicates that both amplifier T cells and B cells are under the control of suppressor cells (64).

The results obtained with PVP have been consistent with Baker's proposal of suppressor and amplifier cells acting in an opposing manner. ALS-treatment of normal mice produced a marked amplification of the PVP-specific PFC response, but ALS-treatment of nude mice was without effect (6). The lack of ALS-induced amplification in nude mice provided additional evidence for the thymus-dependency of amplifier cells (6). Thus, the Con A-induced suppression and amplification of PVP-specific PFC responses may be due to activation of either suppressor

or amplifier T cells when Con A was given at the appropriate time (Figure 1). Con A was without effect in nude mice which may indicate the thymus dependency of both suppressor and amplifier cells (Table 2).

Baker has observed that ALS-treatment of low dose paralyzed mice partially reversed the effects of low dose priming with S-III (49). He attributed the amplified response of ALS-treated, low dose paralyzed mice to the removal of suppressor cells by the ALS. Furthermore, he noticed that the degree of amplification was the same for low dose paralyzed, ALS-treated mice and ALS-treated control mice, although ALS-treated low dose paralyzed mice generated far fewer PFC than ALS-treated control mice. The lower number of PFC in ALS-treated low dose paralyzed mice was attributed to a reduction by suppressor cells in the number of precursor S-III PFC (49). Lake has also shown that low dose paralysis to PVP may be mediated by suppressor cells (48). However, Con A-amplified low dose paralyzed mice responded as well as Con A-amplified control mice (Table 3). Because low dose paralyzed mice responded as well as control mice to the Con A treatment, this result may indicate that the PVP precursor pool was not reduced by the action of the low dose induced suppressor cells.

The role of macrophages in antibody responses to TI antigens is unclear (9-11). Silica, a specific macrophage toxin (66), was given in vivo in an attempt to define the role of macrophages in the immune

response to PVP, a TI antigen. However, instead of the expected decrease in the PVP-specific PFC response, the silica produced an unexpected increase in the PFC response to PVP. A portion of the above study was an attempt to determine the mechanism of silica-induced amplification of the PVP-specific PFC response.

Perhaps the most interesting feature of the study was the experiment summarized in Figure 4. Silica given at various times with respect to immunization with PVP produced two peaks of amplification. One peak of amplification occurred when silica was given on either day -2 or day -1, and the other when silica was given on day +2. A similar biphasic curve was also observed by Weiser and Pearsall when silica was administered at various times with respect to allogeneic skin grafting (75). The significance of the biphasic nature of the curve is unknown.

The results of Table 10 suggested that the amplification produced by silica was thymus dependent. Although silica-treated nude mice responded slightly higher than control nude mice, the difference was not statistically significant. Since this difference was not significant when comparing groups of nude mice, the amplification observed in normal mice was not due to the removal of a population of suppressor macrophages; such suppressor macrophages have been reported in other systems although their relationship with suppressor T cells is unclear.

(111).

There are several possible mechanisms of silica-induced amplification of the PVP-specific PFC response:

- 1) Silica may be directly activating amplifier cells,
- 2) Silica may be removing or interfering with the activity of suppressor cells,
- 3) Amplifier cells may be activated by a product released from lysed macrophages,
- 4) Enzymes released from lysed macrophages may interfere with the activity of suppressor cells,
- 5) Products released from lysed macrophages may have a mitogenic effect on B cells mediated through T cells.

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