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 µg 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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Date 20 September 1978

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ABSTRACT

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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), lipolysaccharide (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 heterogenous 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 Ly-$1^{+}2,3^{-}$. Both cytotoxic effector cells and suppressor cells implicated in regulating antibody production have 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 nonspecific helper T cell replacing factor, called allogeneic effect factor (AEF) (21), the production of which may be dependent on the presence of 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 Ly-$1^+2,3^-$ and Ly-$1^-2,3^+$ T cells to Con A nonspecifically 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 <u>in vitro</u> 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 resistence[1] 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). Aerosal 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 histocompatability 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 acidfied-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 μg 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 μg 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 or 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% Na_2CO_3. 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 Students 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

[a]CON A WAS GIVEN I.V. 2 DAYS AFTER IMMUNIZATION WITH PVP.

[b]ARITHMETIC MEAN ± S.D. OF THE NUMBER OF PVP-SPECIFIC PFC DETERMINED 5 DAYS AFTER IMMUNIZATION WITH 0.25 μg PVP.

[c]MORTALITY 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.

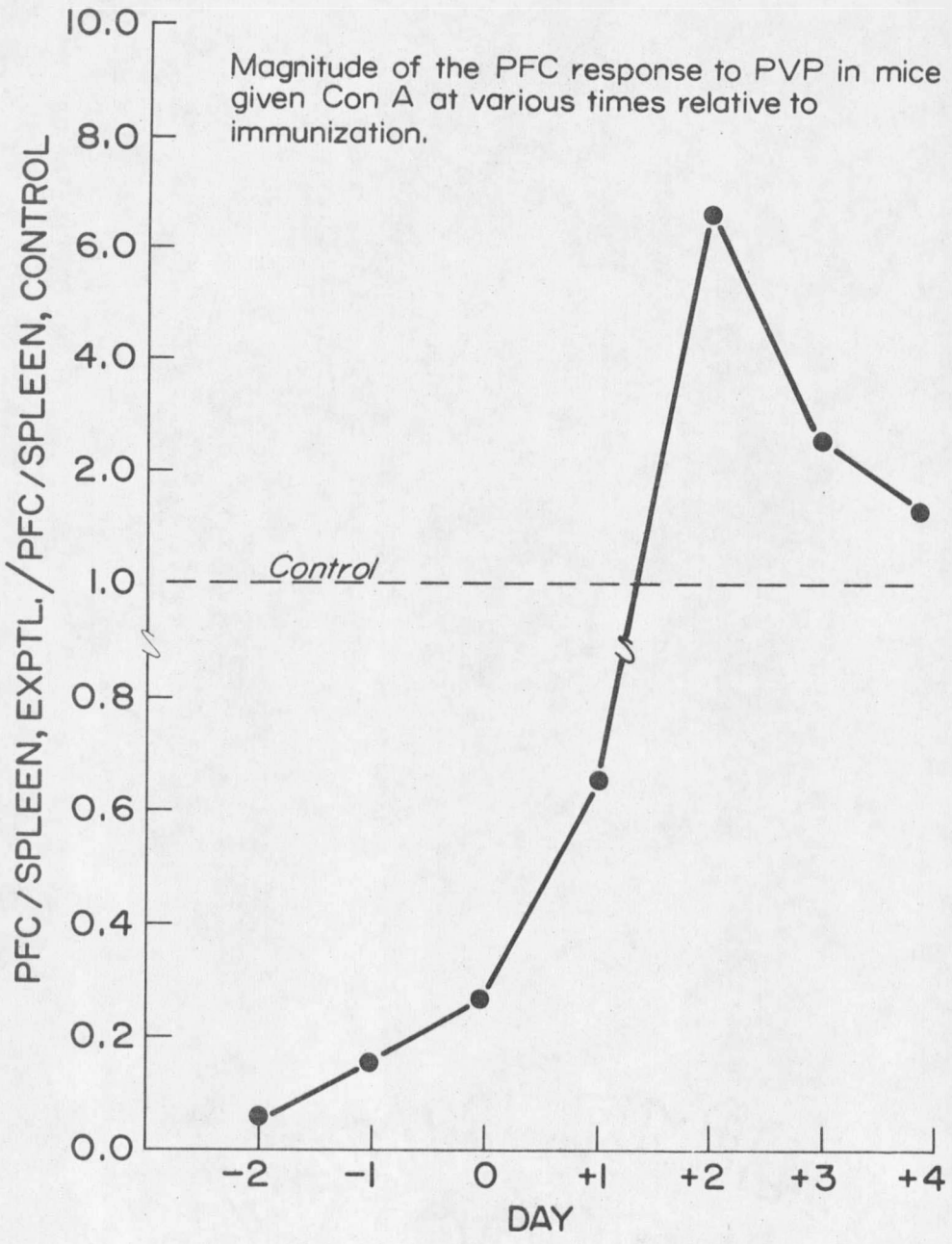
Groups of NLM (Bomholtgard Ltd., Ry, Denmark) were given a single injection of 150 µg of Con A on days -2, -1, 0, +1, +2, and +3 with respect to immunization with PVP. All mice received the optimal immunogenic dose of PVP (0.25 µg) on day 0 and the number of PVP-specific PFC was determined 5 days later. 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 µg Con A i.v. at various times with respect to immunization with 0.25 µg PVP on day 0. Each point represents the mean PFC of 5 mice generated 5 days after immunization with PVP.


Magnitude of the PFC response to PVP in mice given Con A at various times relative to immunization.
PFC/SPLEEN, EXPTL./PFC/SPLEEN, CONTROL
10.0
8.0
6.0
4.0
2.0
1.0
0.8
0.6
0.4
0.2
0.0
Control
−2
−1
0
+1
+2
+3
+4
DAY

(Bomholtgard Ltd., Ry, Denmark) were given 150 µg Con A either on day 0 or day +2 with respect to immunization. All mice received the optimal immunogenic dose of PVP (0.25 µg) 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 µg PVP and treated with 150 µg 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	PVP-SPECIFIC PFC[b] 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

[a]CON A (150 µg) WAS GIVEN I.V. EITHER ON DAY 0 OR DAY +2 WITH RESPECT TO IMMUNIZATION WITH PVP.

[b]ARITHMETIC 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

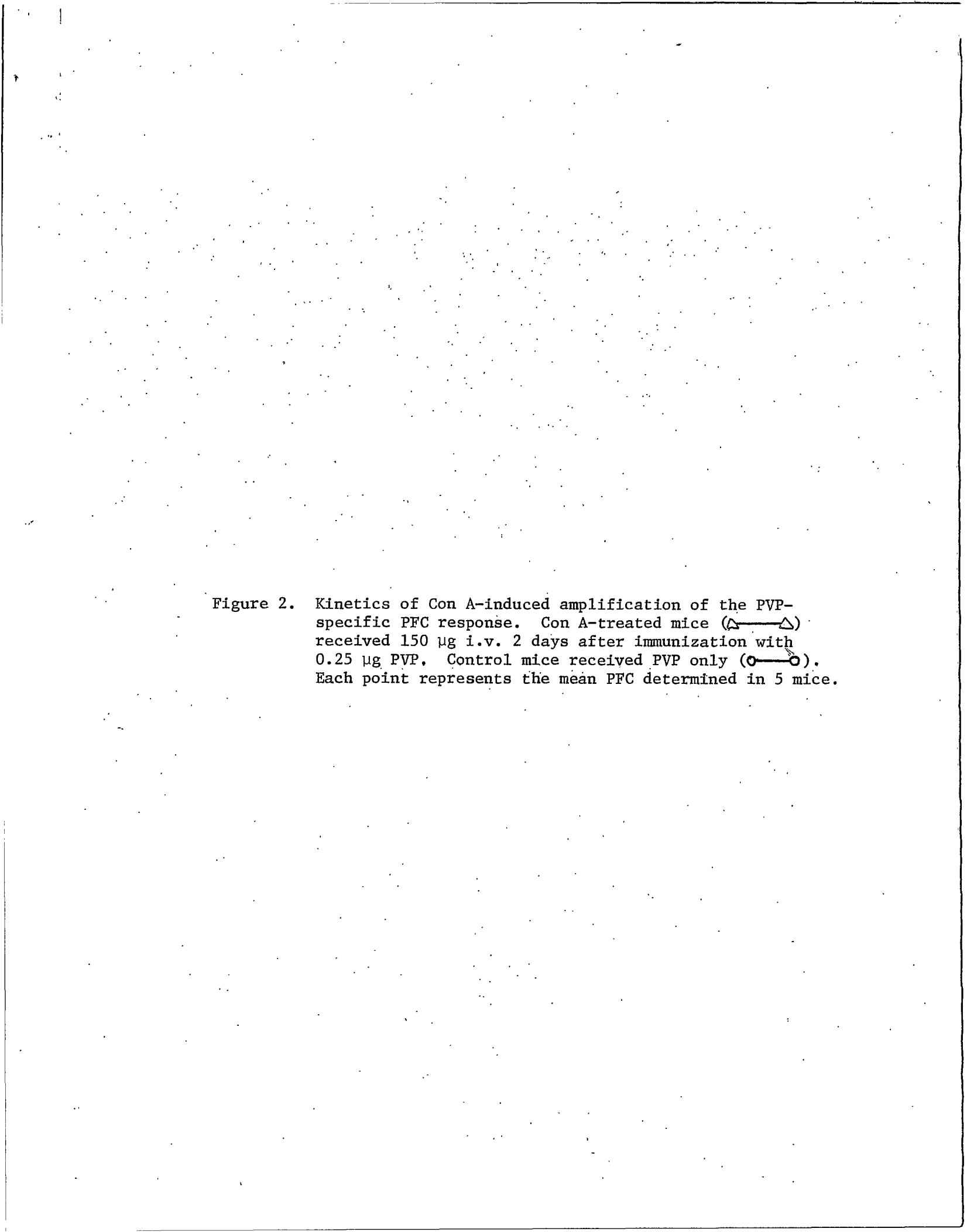


Figure 2. Kinetics of Con A-induced amplification of the PVP-specific PFC response. Con A-treated mice (△——△) received 150 µg i.v. 2 days after immunization with 0.25 µg PVP. Control mice received PVP only (○——○). Each point represents the mean PFC determined in 5 mice.