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INFLUENCE OF CYCLOPHOSPHAMIDE ON THE RESPONSE OF MICE TO BOVINE AND PORCINE TRANSFER FACTOR

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INFLUENCE OF CYCLOPHOSPHAMIDE ON THE RESPONSE OF MICE TO BOVINE AND PORCINE TRANSFER FACTOR

by

Paul Douglas Clark

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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Paul Douglas Clark

entitled Influence of Cyclophosphamide on the Response of Mice to Bovine and Porcine Transfer Factor

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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ABSTRACT

Cyclophosphamide (CY) was injected intraperitoneally into mice at varying dosage levels 48 hours prior to intravenous inoculation of constant amounts of dialysable leucocytic transfer factor (TF). Bovine TF to coccidioidin and Brucella abortus and porcine TF to Mycobacterium avium were used. Twenty-four hours after TF injections, mice were tested for delayed hypersensitivity by footpad inoculation with specific antigens.

The results show that CY slightly enhanced the delayed hypersensitivity response to the specific test antigens in all three systems. This was not true at every CY dose level, however, since there was a correlation between the dosage of CY and the time of peak footpad swelling. Cyclophosphamide at a low level (20 mg/kg body wt) caused the maximal reading at 24 hours after test, whereas the high level dose (200 mg/kg) showed greatest thickening of the footpad at 48 hours.

Transfer factor dosage differences appeard to change the response relatively little, if at all. The cyclophosphamide dose response, on the other hand, showed disparities among various antigen systems and at the

different observation times. Under these conditions, no consistent, striking effects of CY on transfer could be noted.

CHAPTER 1

INTRODUCTION

Cyclophosphamide (CY) was first synthesized by Arnold, Bourseaux and Brock (1) as an antitumor agent. The cytotoxic and immunosuppressive effects of CY depend upon its metabolism by hepatic microsomes to alkylating agents. The major route for activation is believed to be via 4-hydroxycyclophosphamide and aldophosphamide to phosphoramide mustard and nor-nitrogen mustard. Phosphoramide mustard is perhaps the most likely ultimate alkylating metabolite since it alkylates under physiological conditions and is directly cytotoxic (2). It is generally believed that alkylating agents demonstrate their immunosuppressive activity by inhibiting nuclear DNA replication. Cyclophosphamide has been used since the late 1950's to suppress antibody (Ab) formation (3). In the early 1960's, Maguire and Maibach (4) and Salvin and Smith (5) employed CY to study cell-mediated immunity (CMI). However, they studied immunological unresponsiveness without separating the humoral or CMI systems.

Therefore, many reports before 1974 (6, 7) attempted to explain the influence of CY on CMI as a function of the response of the B (Ab) cell to CY. They proposed that the

removal of suppressor B cells or prevention of formation of suppressive Ab was the mechanism by which CY enhanced delayed hypersensitivity (DH) (8, 7).

Histological evidence showed that CY caused depletion of lymphocytes from the non-thymus dependent areas of lymph nodes and spleens, while having little effect on the thymus dependent areas (9, 10, 11). There was also a proportional increase in lymphocytes carrying the theta antigen (9). The target cells were short lived and had a rapid rate of turnover in the tissue. The histological evidence combined with the inhibition of the Ab response by CY argued that the target cell for CY was the B lymphocyte.

Since the early 1970's, however, there has been a growing body of evidence that CY does effect T (CMI) lymphocytes. Cyclophosphamide prolongs the survival of skin allografts. Both T and B cells are needed to restore Ab production in CY treated mice (12). Jokipii and Jokipii (13) with the use of Ars-Tyr, which does not elicit a B cell response, were able to demonstrate that CY suppressed CMI independently of the B cell response. LaGrange, Mackaness and Miller (6) hypothesized that CY must be active against both T and B cells, since administration at different times can destroy either the mediators of DH or the mediators of Ab. Therefore, CY must either eliminate T

cells or some non-specific factors as suggested by the reduction of turpentine irritation by CY treatment (14).

The confusion as to whether CY eliminated T cells or B cells stemmed from the dosage of CY used. The problem was that the commonly used dose of CY, 300 mg/kg, can eliminate both T and B cells. In order to study the effects of CY on CMI it would be necessary to separate the influence of CY on the T cells from the influence of CY on the B cells. Also, it would be necessary to establish the mode of action of CY on T cells.

Polak and Turk (15), in 1974, showed that there is a small pool of T cells sensitive to the action of CY. Subsequently, histological reports provided direct evidence that CY can eliminate T cells (16, 17). Askenase, Hayden and Gershon (18) also provided evidence that CY can effect T cells, but more importantly, they were able to separate the influence of CY on the T cells from the influence of CY on the B cells by controlling the dosage of CY. They discovered that CY was able to augment DH at doses of CY that do not influence Ab titers (200 mg/kg). They also suggested that the enhancement of DH by CY was due to the elimination of suppressor T cells. Other investigators (19, 20, 21) using mice and guinea pigs, also suggested that CY eliminated suppressor T cells. However, it was the work of Mitsuoka, Baba and Morikawa (22), who measured the

time of recovery of different T cell subpopulations, that provided direct evidence that CY can eliminate suppressor T cells.

The target cell of CY is not the mature suppressor, but the suppressor precursor cell. Turk and Poulter (10) had noted that the target cells were short lived and had a rapid turnover in the tissues. LaGrange, Mackaness and Miller (5) showed that the popliteal lymph node proliferative response was delayed for 48 h, yet the effects of CY were long term. Since the cells influenced by CY were susceptible to the effects of CY only at certain stages in cell cycle (23), the target cells must be rapidly dividing precursors. Gill and Liew (24) provided direct evidence that CY inhibits the precursors of suppressor cells. Through the use of time course studies and Ficoll density gradients, they demonstrated that CY inhibited precursor cells, but not mature suppressor cells.

Schwartz, Askenase and Gershon (25) created a model for the action of CY in which the DH Amplifier (Helpers) and Suppressor Amplifier (Precursors) were sensitive to the action of CY. The DH inducer and the Suppressor cells were resistant to CY. Thus, CY is able to enhance DH reactions by eliminating suppressor T precursor cells. It should be noted that the role of B cell suppressors in enhancing DH with CY has not been completely determined (26, 27).

Timing, as well as dosage, of CY is important if DH is to be enhanced. Salvin and Smith (5) noted that CY must be given in advance of the antigen to enhance DH. This observation was confirmed by others (28, 6). Kerckhaert, Hefhuis and Willers (29) studied the effects of variation in time and dose of CY on both Ab and DH. They discovered that not only is the timing and dosage critical but also the route by which CY is injected and, importantly, when the effects of CY are measured. Thus, it is possible to show both suppression and enhancement depending upon the time at which the effects of CY are observed. One of the major criteria of delayed hypersensitivity and cell-mediated immunity is passive transfer from a sensitive donor to a non-sensitive recipient. Cellular transfer was demonstrated by Landsteiner and Chase in 1942 (30).

Transfer of cell-mediated immunity by cell-free extracts was first reported in guinea pigs by Jeter, Tremaine and Seebohm (31) in 1954. Lawrence (32), in 1955, described similar experiments in humans. He later gave the name "Transfer Factor (TF)" to the extracts (31). Transfer factor has been described as a dialyzable leukocytic extract of less than 10,000 molucular weight which contains polypeptide and polynucleotide components, confers long term sensitivity to the leukocytes, and is resistant to DNase, RNase and trypsin (34, 35). It has been reported that TF

is lacking in sulfur-containing amino acids and is heat stable at 56°C for 30 minutes (34). It has the ability to transfer CMI (34, 35) without transferring humoral immunity (33). Transfer factor also can restore the cellular immune system in patients with immuno-deficiency diseases, such as Wiskott-Aldrich syndrome (36).

Studies on other lymphokines, such as migration inhibition factor, have shown a decrease in activity (37, 38) suggesting that CY might also affect the function of TF. Cyclophosphamide enhanced the transfer of DH by whole cells from CY treated donors to non-CY treated recipients (39). Since CY enhances DH reactions by the elimination of suppressor T cell precursors and since TF has the ability to transfer DH reactions, is it possible for CY to enhance the transfer of DH by TF when CY is administered to the recipient animal?

Therefore, our purpose is to administer Bovine TF at a constant dose to mice previously given varying doses of CY.

CHAPTER 2

MATERIALS AND METHODS

Animals

BALB/c and Swiss-Webster mice of both sexes were obtained from colonies in the Department of Microbiology at The University of Arizona. The animals weighed between 20 and 33 grams and were fed a diet of Wayne Lab-Blox. Water was available ad libitum.

Cyclophosphamide

Cyclophosphamide (Cytoxan, MeadJohnson Laboratories, Evansville, Ind.) was reconstituted and diluted with sterile saline and stored at 4C. Mice were injected intraperitoneally (IP) with varying doses of CY 48 h previous to the injection of TF.

Preparation of Transfer Factor

Bovine TF was prepared from approximately 4 L of blood collected from the external jugular vein, using a 13g needle under vacuum, into a flask containing 60 ml of 15% EDTA, pH 7.0. The blood was continually mixed during collection with a magnetic stirrer. It was then packed in ice until it could be dispensed into 250 ml centrifuge bottles and spun at 1,500 x g for 40 min at 4C. After

centrifuging, the plasma was aspirated and the buffy coat was removed using a 10 ml pipet with an aspirator bulb. The buffy coat was then deposited into another 250 ml bottle. The buffy layer from the entire sample was pooled. The pooled cells were centrifuged at 1,900 x g for 50 min at 4C. The residual plasma was aspirated and the pooled buffy was removed with a Pasteur pipet. The buffy was placed in 50 ml centrifuge tubes and spun at 1,000 x g for 50 min at 4C. Once again, the plasma was aspirated, the cells removed with a Pasteur pipet into another 50 ml centrifuge tube. The volume was adjusted with sterile saline and the cells counted by using a hemocytometer or Coulter. A differential count was prepared using Wright's-Giemsa stain, and the total number of lymphocytes calculated. Counts and differential stains were also prepared from the whole blood.

The cells were frozen and thawed 10 times using a dry ice-acetone mixture for freezing and a 37C water bath for thawing. After the last thaw, the broken cells were spun at 20,000 x g at 4C for 30 min. The supernatant fluid was vacuum dialyzed, using dialyzing tubing having a pore radius of 24 angstroms, at 4C. The TF was then filter sterilized, using filters with a diameter of .22u, lyophilized and stored at -20C.

Porcine TF was prepared from lymph nodes collected at the time of slaughter. The nodes were collected in Hanks' Balanced Salt Solution (HBSS) with 20% serum, trimmed of fat, then minced into fine pieces, after which the material was forced through a 20 gauge 60 mesh screen to produce a single cell suspension. The cells were centrifuged at 500 x g for 15 min and resuspended in a measured amount of HBSS without serum. A hemocytometer count was done using an exclusion dye, 0.4% trypan blue, to determine viability (40). The cellular suspension was then disrupted by freezing and thawing, vacuum dialyzed and stored as described above.

Skin Tests

Bovine sensitivity to coccidioidin occurred naturally and was monitored by skin testing in the nuchol area with 0.1 ml undilute coccidioidin (tested with PPD) without preservative (W. T. Northey) and read 48 h later. The pigs were the experimental animals of Dr. Glenn Songer, Department of Veternary Science.

The Foot Pad Swelling Test

The right rear foot pad of each mouse was measured three times immediately after TF injection. The foot pads were measured by a caliper ("Schnelltaster," H. C. Kröplin Co., Germany) graduated in 0.05 mm divisions. Each of the

measurements were recorded. Then the mice were injected with 0.05 ml of the challenge dose (coccidioidin undiluted, M. avium PPD 0.142 mg/ml and Brucella 100 ug/ml) intradermally in the right hind foot pad. The pads were measured at 24 h and at 48 h, taking three measurements each time. The averages from the 24 h and the 48 h readings were then subtracted from the average of the prechallenge readings.

Statistical Analysis

The results are expressed as the arithmetic mean of the foot pad swelling test of the mice in each treatment. A Model 1 anova was performed to analyze the statistical significance of the results.

CHAPTER 3

EXPERIMENTAL RESULTS

A preliminary series of experiments was designed to determine whether treatment of recipient animals with cyclophosphamide forty-eight hours before administering transfer factor enhanced delayed hypersensitivity. For these experiments, CY at 20 or 200 mg/kg was injected 48 h in advance of a constant dose (based on 10^7 lymphocyte equivalents, L. eq.) of bovine transfer factor specific for coccidioidin. Tests of TF preparations showed that 10^7 L. eq. gave a consistent positive response (W. S. Jeter, personal communication). Five mice were used in each group. The CY doses were chosen on the basis that these concentrations of the drug should amplify DH without affecting antibody response (18). Mice were tested by footpad inoculation immediately after TF injection, and swelling was measured at 24 and 48 h after testing.

The results of experiment A (Table 1) show that CY enhanced the DH reaction. The 24 h readings for animals receiving 20 and 200 mg/kg CY show a statistically valid increase over the untreated TF recipient values, but only treatment with CY at 20 mg/kg is statistically significant in comparison with the antigen controls. Cyclophosphamide

Table 1. Influence of cyclophosphamide on passive transfer of delayed hypersensitivity to coccidioidin in mice by bovine dialyzable leucocytic extracts.

CY Dose[b] mg/kg	Experiment A[a] Footpad Swelling (0.1 mm)[c] 24 h Avg.	24 h Extremes	48 h Avg.	48 h Extremes
20	3.8[d]	3.3-4.2	1.6	1.0-2.0
200	2.4[d]	1.2-3.7	2.4	1.6-3.7
0	0.5	0.3-0.8	1.2	0.3-2.2
Antigen Control	1.4	0.9-1.9	0.2	-0.3-0.5
	Experiment B[a]			
20	2.3	1.8-3.1	1.6	1.3-2.0
200	1.0	0.2-2.0	1.7	1.1-2.3
0	2.6	2.0-4.0	2.8	2.1-3.2
Antigen Control	1.7	1.3-3.1	0.7	0.2-2.3

[a]Transfer Factor Dose = 10^7 Lymph. eq. in 0.2 ml intravenously, Swiss-Webster mice, 5 animals/group

[b]Cyclophosphamide injected intraperitoneally, 48 h before TF

[c]Undilute coccidioidin, 0.5 ml intradermally

[d]$p < 0.05$

treatment at 20 mg/kg was also statistically different from CY at 200 mg/kg. When comparing the 24 h readings to the 48 h readings, animals receiving CY at 200 mg/kg had the same readings for both time periods. In contrast, CY given at 20 mg/kg dropped from 3.8 to 1.6, whereas the TF control readings increased from 0.5 to 1.2. The antigen control dropped from 1.4 to 0.2.

At 48 h both CY readings were greater than the non-CY TF reading, but not at a significant level. Cyclophosphamide at 200 mg/kg at 48 h increased in value over CY at 20 mg/kg. Both doses of CY were at a significantly statistical level over the antigen controls, but the non CY TF is not statistically valid over the antigen controls.

The second experiment (experiment B) was disappointing in that CY at both doses failed to enhance the readings over the non CY TF reading. This was true at both time periods. However, cyclophosphamide at 20 mg/kg at 24 h. The only value at 24 h which was statistically valid was in the CY at 200 mg/kg group, which was a suppression compared to the non CY TF reading. At 48 h the only statistically valid increase was the non CY TF reading over the antigen controls. The CY readings, although greater than the antigen control values, were not statistically valid.

One factor which could account for the different results in Experiments A and B was the fact that TF

preparations used were prepared from blood collected from the donor cow on two different days ten months apart.

Two experiments repeating Experiments A and B and using the same numbers of mice, but employing TF prepared from cells collected on different dates from the donor cow, gave similar results. Since statistical analysis was inconclusive using these numbers, our next experiment (C) utilized 30 animals per group with a constant dose of coccidioidin TF (10^7 L. eq.) and low (20 mg/kg) and high (200 mg/kg) doses of CY. The results (Table 2) showed a statistically significant increase in footpad thickness in the low dose group at 48 h. The antigen control values were unusually high (2.5 units) at 24 h, whereas the high CY dose animals showed a diminished response (0.8 units) when compared with the TF controls (1.4).

Since no striking differences were noted among the groups and since the action of CY is considered to be dose dependent, we elected to do a dose response experiment. Coccidioidin TF at a constant dose of 10^7 L. eq. and CY doses ranging from 10 mg/kg to 400 mg/kg were employed. The results (Table 3) show that only one dose of CY (100 mg/kg) caused a significantly different reading when compared to the untreated TF recipients. Cyclophosphamide at 20 and 200 mg/kg doses effected a slight increase in footpad swelling, whereas the CY at 50 mg/kg group showed a

Table 2. Influence of cyclophosphamide on passive transfer of delayed hypersensitivity to coccidioidin in mice by bovine dialyzable leucocytic extracts.

CY Dose[b] mg/kg	Experiment C[a] Footpad Swelling (0.1 mm)[c]			
	24 h		48 h	
	Avg.	Extremes	Avg.	Extremes
20	1.6	-0.2-3.4	3.0[d]	1.7-4.5
200	0.8	-0.3-6.3	2.2	-0.1-4.0
0	1.4	0.2-4.2	1.9	-0.5-3.7
Antigen Control	2.5	0.7-3.2	0.3	-0.8-1.3

[a]Transfer Factor Dose = 10^7 Lymph. eq. in 0.2 ml intravenously, Swiss-Webster mice, 30 animals/group except for antigen control, 10 animals/group

[b]Cyclophosphamide injected intraperitoneally, 48 h before TF

[c]Undilute coccidioidin, 0.05 ml intradermally

[d]$p < 0.05$

Table 3. Influence of cyclophosphamide on passive transfer of delayed hypersensitivity to coccidioidin in mice by bovine dialyzable leucocytic extracts.

CY Dose[b] mg/kg	Experiment D[a] Footpad Swelling (0.1 mm)[c]			
	24 h		48 h	
	Avg.	Extremes	Avg.	Extremes
10	0.5	0.1-0.9	-0.6	-1.3-0.2
20	2.9	2.2-3.5	0.5	-0.5-1.2
50	2.5	1.3-3.2	1.3	0.6-1.8
100	4.1[d]	2.5-5.2	1.3	0.5-2.2
200	2.8	2.3-3.5	1.4	1.1-1.8
300	1.4	0.2-2.7	1.5	0.3-2.0
400	1.6	1.0-1.8	0.7	0.3-1.0
0	2.7	2.0-3.6	1.9	1.0-2.7
Antigen Control	2.7	2.3-2.9	2.2	1.8-2.8

[a]Transfer Factor Dose = 10^7 Lymph. eq. in 0.2 ml intravenously, Swiss-Webster mice, 5 animals/group

[b]Cyclophosphamide injected intraperitoneally, 48 h before TF

[c]Undilute coccidioidin, 0.05 ml intradermally

[d]$p < 0.05$

slight suppression of response. Cyclophosphamide at 300 and 400 mg/kg markedly inhibited the footpad thickening, with the reading on the 300 mg/kg group showing significant depression at $P < 0.05$. The mean value for CY treated group at 10 mg/kg was significantly inhibited ($P < .001$). It was only slighly above baseline readings.

The 48 h readings dropped well below the 24 h levels in all cases. It should be mentioned that the antigen control animals in this experiment gave unusually high values, perhaps reflecting some toxicity for these animals of this coccidioidin preparation.

Although the results in the coccidioidin studies indicated both suppressive and enhancement effects by cyclophosphamide at different dosage levels, the question arose whether these values could be amplified. Therefore, in the next experiments, transfer factor to a different antigen (Mycobacterium avium), from a different species (porcine) and in an inbred mouse strain (BALB/c) was tried. The results of these experiments are given in Table 4. In Experiment E, both doses of CY enhanced the DH reaction, but not at a significant level. There was no difference between the readings in the CY treated groups at either 24 h or 48 h. These values were statistically valid in comparison with the antigen control group. At 24 h, the TF control group was not valid, whereas this

Table 4. Influence of cyclophosphamide on passive transfer of delayed hypersensitivity to Mycobacterium avium in mice by porcine dialyzable leucocytic extracts.

CY Dose[b] mg/kg	Experiment E[a] Footpad Swelling (0.1 mm)[c]			
	24 h		48 h	
	Avg.	Extremes	Avg.	Extremes
20	3.5	2.8-3.5	2.9	2.1-3.8
200	3.3	2.7-3.8	3.0	1.9-3.8
0	2.5	2.2-2.7	3.0	2.5-3.7
Antigen Control	1.4	0.6-2.0	0.8	-0.2-1.5

[a]Transfer Factor Dose = 10^7 Lymph. eq. in 0.2 ml intravenously, BALB/c mice, 5 animals/group

[b]Cyclophosphamide injected intraperitoneally, 48 h before TF

[c]M. avium PPD 142 ug/ml, 0.05 ml intradermally

situation had changed at 48 h, probably due to a sharp diminution in the nonspecific inflammatory response to the antigen. Once again, there was a question concerning the size of the animal groups. Therefore, two experiments, one employing Swiss-Webster mice (Table 5, Experiment F) and another using BALB/c animals (Table 5, Experiment G) were done with groups of 10 subjects. In Experiment F, the only significant result came at 48 h, when the animals treated with 200 mg/kg of cyclophosphamide gave readings considerably above the other groups. In Experiment G, no valid changes were observed in any of the groups.

In the light of these inconclusive results, we elected to do a cyclophosphamide dose response in the M. avium system with Swiss-Webster mice. The transfer factor dose was halved. The "lethal" CY doses of 300 and 400 mg/kg were eliminated. The results (Experiment H) are shown in Table 6. At 24 h, CY treated groups at 10, 50 and 100 mg/kg showed a significant increase over the control groups. At 48 h, on the other hand, the TF controls, 200 mg/kg and 50 mg/kg groups had increased statistically, whereas the others had decreased the 100 mg/kg group sharply. Cyclophosphamide at 20 mg/kg recorded a slight increase. Thus, these results suggest that there is no clear-cut influence by CY on transfer of DH under a variety of experimental circumstances.

Table 5. Influence of Cyclophosphamide on passive transfer of delayed hypersensitivity to Mycobacterium avium in mice by porcine dialyzable leucocytic extracts.

CY Dose[b] mg/kg	Experiment F[a] Footpad Swelling (0.1 mm)[c] 24 h Avg.	24 h Extremes	48 h Avg.	48 h Extremes
20	1.9	0.7-3.2	0.3	-0.8-2.8
200	2.3	0.9-4.0	1.2[d]	0.2-1.5
0	1.4	0.2-3.0	-0.3	-1.5-0.3
Antigen Control	1.5	1.0-2.2	-0.1	-0.3-0.2
	Experiment G[a]			
20	0.3	-1.5-2.5	-0.2	-1.2-1.5
200	0.3	-2.1-1.5	0.3	-1.3-1.9
0	1.4	0.0-2.9	0.1	-0.7-0.5
Antigen Control	1.9	1.2-2.5	-0.4	-0.5--0.2

[a]Transfer Factor Dose = 10^7 Lymph. eq. in 0.2 ml intravenously, Experiment F Swiss-Webster mice, Experiment G BALB/c mice, 10 mice/group except for antigen control, 5 mice/group

[b]Cyclophosphamide injected intraperitoneally, 48 h before TF

[c]M. avium PPD 142 ug/ml, 0.05 ml intradermally

[d]$p < 0.05$

Table 6. Influence of cyclophosphamide on passive transfer of delayed hypersensitivity to Mycobacterium avium in mice by porcine dialyzable leucocytic extracts.

CY Dose[6] mg/kg	Experiment H[a] Footpad Swelling (0.1 mm)[c] 24 h Avg.	24 h Extremes	48 h Avg.	48 h Extremes
10	3.5[d]	2.6-5.0	2.1	1.5-2.8
20	1.8	1.1-2.7	2.6	1.7-3.3
50	2.9[d]	2.3-3.3	4.4	3.5-5.6
100	2.3[d]	0.5-3.6	0.8	0.0-1.2
200	-0.3	-0.7-0.2	2.2	1.7-2.7
0	0.8	0.2-1.9	3.7	3.5-4.4
Antigen Control	0.4	0.2-0.7	0.2	-1.2-0.5

[a]Transfer Factor Dose = 5 x 10^6 Lymph. eq. in 0.2 ml intravenously Swiss-Webster mice, 5 animals/group

[b]Cyclophosphamide injected intraperitoneally, 48 h before TF

[c]M. avium PPD 142 ug/ml, 0.05 ml intradermally

[d]$p < 0.05$

Considering that the variability could be related to the antigen system tested, a final experiment was done employing bovine transfer factor to Brucella abortus in Swiss-Webster mice. Once again, only one significant value (Table 7) was noted, that in the 20 mg/kg CY treated group at the 48 h observation time. Thus, a consistent, clear-cut CY effect on transfer was again not noted. It should be noted that a CY control where the mice received CY but no TF, then pad tested, gave a minimal swelling of the foot pad at 24 h and no discernible effects at 48 h in all experiments.

Table 7. Influence of cyclophosphamide on passive transfer of delayed hypersensitivity to Brucella abortus in mice by bovine dialyzable leucocytic extracts.

CY Dose[b] mg/kg	Experiment I[a] Footpad Swelling (0.1 mm)[c] 24 h Avg.	24 h Extremes	48 h Avg.	48 h Extremes
20	3.5	2.6-4.6	2.9[d]	1.8-4.4
100	2.6	1.7-3.2	1.9	1.0-2.6
200	3.5	2.2-4.5	2.0	1.0-3.2
0	2.7	2.6-3.0	1.1	0.8-1.5
Antigen Control	1.5	0.0-3.0	1.7	0.5-3.2

[a]Transfer Factor Dose = 10^7 Lymph. eq. in 0.2 ml intravenously, Swiss-Webster mice, 5 animals/group

[b]Cyclophosphamide injected intraperitoneally, 48 h before TF

[c]Brucella antigen 100 ug/ml, 0.05 ml intradermally

[d]$p < 0.05$

CHAPTER 4

DISCUSSION

Initially, we expected to ascertain a dosage level of CY in mice which would diminish suppressor T lymphocytes function without as striking an effect on effector cells (20), thereby enhancing TF activity. The inconsistency of the magnitude of response to TF in the presence of CY and the lack of a clean-cut dose dependency relationship were problematical, however. These irregularities seem to be independent of the species source or antigen specificity of the TF employed.

The results indicate that CY enhances the DH response of mice to bovine and porcine TF, when the footpad swelling reaction is used as an indicator. The increases and some depressive reactions do not fit into a clearly recognizable pattern, making it hard to evaluate the influence of CY on the response of mice to TF. Cyclophosphamide did cause statistically valid increases in all three test systems, but not consistently in each experiment.

Since the mechanism of CY action in these experiments is not known, there are several possibilities for the mode of action. Cyclophosphamide may be eliminating the suppressor precursor or helper cell as in the proposed

model of Schwartz, Askenase and Gershon (25), therefore acting as a positive immunoregulatory mechanism as reported by Dwyer, Parker and Turk (39). Also, changes in lymphocyte function other than cell death have been reported by Balow, Parrillo and Fauci (38). These changes markedly depressed blastogenesis, depressed migration inhibitory factor and depressed cytotoxicity reactions. Therefore CY might be enhancing effector cell activity while concurrently depressing other lymphokine activity. The question of dosage dependency becomes a crucial one in this case, however.

It is conceivable that a different route of administration of the CY might have altered the results. We elected to use the intraperitoneal path. Some other workers have chosen the intravenous method.

As in the case of the CY, the dose response to the TF does not appear to follow a mathematical pattern. Whether increased (or diminished) doses of TF in the presence of varying amounts of CY might amplify the responses observed here is a question for speculation.

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