# IMMUNE RESPONSE OF MALLARD DUCKS TREATED WITH IMMUNOSUPPRESSIVE AGENTS: ANTIBODY RESPONSE TO ERYTHROCYTES AND IN VIVO RESPONSE TO PHYTOHEMAGGLUTININ-P

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ABSTRACT: The ability of two in vivo tests to assay immune competence of mallard ducks (*Anas platyrhynchos*) treated with various immunomodulatory agents was examined. Skin responses to phytohemagglutinin-P (PHA-P) injected intradermally and serum antibody levels produced in response to sheep red blood cells (SRBC) were measured. As measured by the skin response to PHA-P, ducks injected intramuscularly with cyclophosphamide or cyclosporine did not respond differently from control-injected ducks. Dexamethasone injected intramuscularly significantly suppressed the skin response to PHA-P. As measured by antibody levels in response to SRBC, ducks injected intramuscularly with cyclophosphamide responded with antibody titers similar to controls. Cyclosporine injected intramuscularly reduced the level of immunoglobulin (Ig) G significantly in one of two experiments. Dexamethasone injected intramuscularly reduced peak total and IgG titers. These experiments provide information on the viability of these two in vivo tests to reflect immune competence of mallard ducks.

*Key words:* Immune response, mallard ducks, *Anas platyrhynchos*, antibody, mitogen, cyclophosphamide, cyclosporine, dexamethasone, immune suppressants, experimental study.

## INTRODUCTION

Concern over possible sublethal effects of environmental contaminants has alerted managers and researchers of the need to examine the status of the immune system of waterfowl. Many environmental contaminants have been found to affect the immune system: pesticides such as DDT, dieldrin, carbaryl, carbofuran, methylparathion, maneb, chlordane, and hexachlorobenzene: heavy metals such as lead, cadmium, nickel, chromium; organometals; and halogenated hydrocarbons such as PCB, PBB, TCDD, and others (Lu, 1985; Dean et al., 1986). Some of these substances have been found in tissues of waterfowl (White, 1979; Parslow et al., 1982; DiGuillio and Scanlon, 1984; Foley and Batcheller 1988; King and Krynitsky, 1986; Prouty and Bunck, 1986; Mora et al., 1987) and other birds. The interaction between some of these substances and disease resistance of mallard ducks has been studied (Friend and Trainer, 1970, 1974a, b). Methods are needed to evaluate the health of the immune system of waterfowl. These methods must be easily applied to field investigations where an indicator species such as the ubiquitous mallard can be used.

Numerous techniques are available to assess the immune response of animals. These assays typically have been developed to study the immune response of laboratory animals. Modifications may be necessary to apply these techniques to wildlife species and obtain meaningful results. Also, results of an assay cannot always be interpreted similarly among species (Schultz, 1982). One in vivo immune function test that has been used for avian species (Stadecker et al., 1977; McCorkle et al., 1980; Cook and Springer, 1983; Tsiagbe, 1987a, b) and may be applicable to waterfowl is the T-cell dependent, in vivo skin response to phytohemagglutinin-P (PHA-P). Another in vivo function test that

has been extensively used for avian species is the antibody response to heterologous erythrocytes (i.e., chicken or sheep red blood cells) (Sato and Glick, 1970; Gross and Siegel, 1973; Gross et al., 1980; Toth and Norcross, 1981a, b; Tsiagbe et al., 1987a, b).

We investigated the viability of these two tests to evaluate the competence of the immune system of mallard ducks. To determine the ability of these tests to assess the immune status of ducks, mallards were treated with three known immunosuppressive agents (cyclophosphamide, cyclosporine, and dexamethasone) injected intramuscularly (i.m.). Cyclophosphamide is a synthetic antineoplastic drug which is cytotoxic for lymphocytes. It has been shown to decrease humoral immune responses of a variety of young avian species including Pekin ducklings (Hashimoto and Sugimura, 1976). Cyclosporine is a non-cytotoxic fungal metabolite that was shown to suppress humoral immune responses in chickens (Pardue et al., 1985), presumably through its effects on T-cell helper activity (White and Shaw, 1986). The synthetic corticosterone, dexamethasone, has also been shown to suppress humoral immune responses in avian species (Gross and Siegel, 1973). Levels of the immune suppressive drugs used in the following studies were based on published values shown to cause immune suppression in avian species without being lethal.

## MATERIALS AND METHODS

### Experimental designs

Seven- to 9-mo-old mallard ducks (*Anas platyrhynchos*) which were reared at the University of Wisconsin (Madison, Wisconsin 53706, USA) from eggs obtained from a game farm (Pine Cone Farms, Park Rapids, Minnesota, 56470, USA) were used in all experiments. During the experimental periods, ducks were housed in individual cages in an environmentally controlled chamber (Biotron, Madison, Wisconsin 53706, USA). Temperature was held at 21 C. Lights were on 12 hr/day except in Experiment five in which lights were on 11 hr/day. A corn-soy maintenance diet prepared at the University of Wisconsin (Madison, Wisconsin 53706, USA) and water were provided ad libitum. These con-

ditions were selected to minimize environmental effects on the antibody response of ducks (Cheville, 1979; Gross and Siegel, 1980).

*Experiment one:* Thirty male ducks were acclimated for 3 wk in the chamber prior to treatment. Average weight of the ducks was 1.5 (0.2) kg at the start of the experiment. Ten ducks were assigned to each treatment. Ducks were injected i.m. with the immunosuppressive agents 2 and 1 days prior to injection (as described below) of the mitogen, PHA-P, the day of mitogen injection, and 1 day after mitogen injection (on days −2, −1, 0, and 1) as follows: (1) physiological saline control (2.6 ml); (2) 25 mg/kg-bw cyclophosphamide in 2.6 ml physiological saline (E.S.I.; Elkins-Sinn, Inc., Cherry Hill, New Jersey 08034, USA); or (3) 3.7 mg/kg-bw dexamethasone (2.6 ml injectable; Phoenix Pharmaceutical, St. Joseph, Missouri 64501, USA). The stimulation index was determined at 12, 24, and 48 hr after mitogen injection as described below.

*Experiment two:* Twenty-four female ducks were acclimated for 1 wk in the chamber prior to treatment. Average weight of the ducks was 1.0 (0.1) kg at the start of the experiment. Eight ducks were assigned to each treatment. Each duck within a treatment was injected i.m. with the immunosuppressive agents two and one day prior to mitogen injection and the day of mitogen injection (on days −2, −1, and 0) as follows: (1) physiological saline control (1.9 ml); (2) 1.5 mg/kg-bw dexamethasone (0.75 ml injectable); or (3) 3.8 mg/kg-bw dexamethasone (1.9 ml injectable). The stimulation index was determined 24 hr after mitogen injection as described below.

*Experiment three:* Thirty-two male ducks were acclimated for at least 1 wk in the chamber prior to treatment. Average weight of the ducks was 1.2 (0.2) kg at the start of the experiment. Eight ducks were assigned to each treatment. Each duck within a treatment was injected i.m. with the immunosuppressive agents 2 and 1 day prior to mitogen injection and on the day of mitogen injection (on days −2, −1 and 0) as follows: (1) physiological saline control (2.5 ml); (2) 7.7 mg/kg-bw cyclosporine (0.1 ml oral solution); (3) 1.5 mg/kg-bw dexamethasone (1.0 ml injectable); or (4) 3.8 mg/kg-bw dexamethasone (2.5 ml injectable). The stimulation index was determined at 24 hr after mitogen injection as described below.

*Experiment four:* Thirty-six female ducks were acclimated in the environmental chamber for 3 wk prior to sheep red blood cell (SRBC) inoculation. Average weight of the ducks was 1.1 (0.1) kg at the start of the experiment. Twelve ducks were assigned to each treatment. Ducks were injected i.m. as follows on days −1, 1, 3, 5, and 7 of SRBC inoculation: (1) physiological

saline control (3 ml); (2) 9.1 mg/kg-bw cyclosporine (0.1 ml oral solution); or (3) 5.5 mg/kg-bw dexamethasone (3 ml injectable). Antibody levels to SRBC were determined on days 0, 4, 6, and 8 of SRBC inoculation as described below.

*Experiment five:* Thirty two ducks were acclimated in the environmental chamber for 3 wk prior to SRBC inoculation. Average weight of the ducks was 1.1 (0.1) kg at the start of the experiment. Six females and two males were assigned to each treatment. Ducks were injected i.m. with treatments as follows 1 to 2 hr prior to (day 0), and 1, 3, 5, and 7 days after (days 1, 3, 5, and 7, respectively) inoculation with SRBC: (1) physiological saline control (1.6 ml) on days 0, 1, 3, 5, and 7 of antigen inoculation; (2) 20 mg/kg-bw cyclophosphamide (in 1.6 ml physiological saline) on days 0, 1, 3, and 5 of antigen inoculation; (3) 9.9 mg/kg-bw cyclosporine (0.1 ml oral solution) on days 0, 1, 3, 5 and day 7 of antigen inoculation; or (4) 5.6 mg/kg-bw dexamethasone (3 ml injectable) on days 0, 1, 3, 5 and 5.0 mg/kg-bw on day 7 of antigen inoculation. Antibody levels to SRBC were determined on days 0, 4, 6 and 8 of SRBC inoculation as described below. On day 7 after SRBC inoculation, the PHA-P skin test was initiated. The stimulation index was determined at 24 and 48 hr after mitogen injection as described below which was also day 8 and 9, respectively, after SRBC inoculation.

### Immune measures

*Skin response:* Ducks were injected in one wing web with the mitogen, 0.2 ml of 1 mg/ml PHA-P (Difco, Detroit, Michigan 48232, USA) in a phosphate buffered saline (PBS) solution, after plucking and marking the injection site. The opposite wing web (control) was injected with 0.2 ml of PBS. The thickness of the wing web at the injection site was measured with a pressure-sensitive micrometer (Dyer, Lancaster, Pennsylvania 17604, USA) just prior to injection of PHA-P (time 0) at 24 hr and other times after injection of PHA-P as indicated. The PHA-P stimulation index was calculated as the difference between the increase in skin thickness from time 0 due to PHA-P and increase from time 0 due to PBS.

*Antibody production:* Ducks were injected intraperitoneally (i.p.) with 2 ml of a 5% suspension (vol/vol) of packed SRBC in PBS. Just prior to challenge, on day 6 after SRBC inoculation, and on other days as previously indicated, samples of blood were collected from the jugular vein to evaluate primary hemagglutinating antibody responses. Blood samples were allowed to stand at room temperature for 1 to 2 hr. Samples were then centrifuged at 750 $g$

for 20 min. The sera were decanted and frozen until serological determinations were performed. Antibody levels to SRBC were determined by a microhemagglutination assay technique (Tsiagbe et al., 1987a, b). The 2-mercaptoethanol (2-Me, Sigma Chemical, St. Louis, Missouri 63178, USA) resistant antibody levels, immunoglobulin (Ig) G, were determined by incubating the serum with an equal volume (50 $\mu$l) of 1.4% (vol/vol) 2-Me in PBS at 37 C for 30 min prior to the hemagglutination test. The 2-mercaptoethanol-sensitive antibody levels (IgM) were determined by subtracting the 2-Me-resistant antibody titer from the total antibody titer (Delhanty and Solomon, 1966). Even though 2-Me-resistant and 2-Me-sensitive titers are not absolute indicators of IgG and IgM levels, IgG and IgM terms are used for brevity. The antibody titers are expressed as the log$_2$ of the highest dilution of serum that agglutinated an equal volume of 0.5% red blood cells.

*Hematology:* Total white blood cell counts, white cell differentials, and percent packed red cell volume was determined on a subset ($n = 3$ or 4/treatment) of the birds in Experiment five. Blood was collected on day 4 or day 8 of SRBC inoculation during Experiment five for these analyses. Total white blood cell counts were determined by the indirect method and differential leucocyte percentages were determined by direct counting as recommended by Dein (1984). Packed red cell volume was determined by withdrawing a sample into a heparinized microhematocrit tube, centrifuging the tube for 5 min in a hematocrit centrifuge, and reading percent volume of packed red cells.

### Statistical analysis

We subjected stimulation indices and antibody titers to an analysis of variance by using the general linear model (Statistical Analysis Systems Institute, Inc. 1982). We determined mean treatment differences by using least square differences.

### RESULTS

#### In vivo mitogen response

Results of the skin tests are reported in Table 1. Dexamethasone lowered the in vivo response to PHA-P in all experiments in which it was used as a treatment. In Experiment one, the group injected with dexamethasone responded with lower stimulation indices at 12 ($P < 0.01$), 24 ($P < 0.05$) and 48 ($P < 0.05$) hr following the injection of PHA-P relative to the control group at the respective times. The 24 hr stimulation index was significantly low-

TABLE 1.   The effect of various immunosuppressive agents on the skin response to phytohemagglutinin-P at different times after stimulation.

| Experiment | Treatment | Stimulation index × 10⁻² mm (standard error) | | |
|---|---|---|---|---|
| | | 12 hr | 24 hr | 48 hr |
| 1 | Control[a] | 120 (14) | 123 (11) | 100 (12) |
| | Cyclophosphamide[b] | 67 (13)* | 93 (18) | 109 (18) |
| | Dexamethasone[c] | 51 (9)* | 80 (17)* | 49 (11)* |
| 2 | Control[d] | | 209 (31) | |
| | Dexamethasone[e] | | 80 (9)* | |
| | Dexamethasone[f] | | 74 (17)* | |
| 3 | Control[g] | | 155 (19) | |
| | Cyclosporine[h] | | 133 (16) | |
| | Dexamethasone[i] | | 71 (12)* | |
| | Dexamethasone[j] | | 61 (8)* | |
| 5 | Control[k] | | 133 (18) | 113 (16) |
| | Cyclophosphamide[l] | | 123 (16) | 156 (23) |
| | Cyclosporine[m] | | 142 (25) | 113 (15) |
| | Dexamethasone[n] | | 59 (11)* | 104 (12) |

* Significant difference from control at $P < 0.05$.
[a] 2.6 ml physiological saline on days −2, −1, 0 and 1 of mitogen injection.
[b] 25 mg/kg-bw cyclophosphamide (in 2.6 ml physiological saline) on days −2, −1, 0, and 1 of mitogen injection.
[c] 3.7 mg/kg-bw dexamethasone (2.6 ml injectable) on days −2, −1, 0, and 1 of mitogen injection.
[d] 1.9 ml physiological saline on days −2, −1, and 0 of mitogen injection.
[e] 1.5 mg/kg-bw dexamethasone (0.75 ml injectable) on days −2, −1, and 0 of mitogen injection.
[f] 3.8 mg/kg-bw dexamethasone (1.9 ml injectable) on days −2, −1, and 0 of mitogen injection.
[g] 2.5 ml physiological saline on days −2, −1, and 0 of mitogen injection.
[h] 7.7 mg/kg-bw cyclosporine (0.1 ml oral solution) on days −2, −1, and 0 of mitogen injection.
[i] 1.5 mg/kg-bw dexamethasone (1.0 ml injectable) on days −2, −1, and 0 of mitogen injection.
[j] 3.8 mg/kg-bw dexamethasone (2.5 ml injectable) on days −2, −1, and 0 of mitogen injection.
[k] 1.6 ml physiological saline on days −7, −6, −4, −2, and 0 of mitogen injection.
[l] 20 mg/kg-bw cyclophosphamide (in 1.6 ml phyisological saline) on days −7, −6, −4, −2, and 0 of mitogen injection.
[m] 9.9 mg/kg-bw cyclosporine (0.1 ml oral solution) on days −7, −6, −4, −2, and 0 of mitogen injection.
[n] 5.6 mg/kg-bw dexamethasone (3 ml injectable) on days −7, −6, −4, −2, and 5.0 mg/kg-bw on day 0 of mitogen injection.

er ($P < 0.01$) for both dose levels of dexamethasone in experiments two and three. Dexamethasone also decreased ($P < 0.05$) the 24 hr but not the 48 hr index in Experiment five.

Cyclophosphamide had no effect on the skin response to PHA-P in Experiments one and five except in Experiment one where the group injected with cyclophosphamide responded with lower stimulation indices at 12 hr after mitogen injection ($P < 0.01$). Cyclosporine also had no effect on the in vivo response to PHA-P in Experiments three and five.

### In vivo antibody response

Results of the antibody tests are reported in Table 2. Dexamethasone reduced peak total and IgG antibody levels in response to SRBC in both experiments. Dexamethasone significantly reduced the day 4 ($P < 0.05$), day 6 ($P < 0.01$), and day 8 ($P < 0.01$) total antibody levels and the day 6 ($P < 0.01$) and day 8 ($P < 0.05$) IgG levels in Experiment four. Dexamethasone decreased the day 6 total and IgG antibody levels ($P < 0.05$) in Experiment five.

Cyclosporine significantly reduced the day 6 and day 8 IgG levels ($P < 0.05$) in Experiment four. Cyclosporine had no effect on the antibody levels in Experiment five.

Antibody levels of cyclophosphamide-treated ducks were not significantly different from control ducks in Experiment five except that the day 4 total antibody level was significantly ($P < 0.05$) lower than the control level.

TABLE 2.   The effect of various immunosuppressive agents on antibody response to Sheep Red Blood Cells (SRBC) before and 4, 6, and 8 days after inoculation.

| Experiment | Treatment | Antibody titers (standard error) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Day 0 | | | Day 4 | | | Day 6 | | | Day 8 | | |
| | | Total | IgG | IgM | Total | IgG | IgM | Total | IgG | IgM | Total | IgG | IgM |
| 4 | Control[a] | 2.9 (0.3) | 0.1 (0.1) | 2.9 (0.2) | 6.7 (0.5) | 1.4 (0.4) | 5.3 (0.3) | 9.1 (0.5) | 5.6 (0.5) | 3.6 (0.3) | 8.8 (0.4) | 5.2 (0.4) | 3.6 (0.2) |
| | Cyclosporine[b] | 2.5 (0.2) | 0 (0) | 2.5 (0.2) | 6.2 (0.7) | 1.7 (0.4) | 4.5 (0.5) | 7.5 (0.7) | 3.1 (0.8)* | 4.4 (0.4) | 7.3 (0.5) | 3.5 (0.7)* | 3.5 (0.6) |
| | Dexamethasone[c] | 2.6 (0.4) | 0 (0) | 2.6 (0.4) | 4.6 (0.5)* | 0.5 (0.2) | 4.2 (0.4) | 6.0 (0.6)* | 2.3 (0.6)* | 3.7 (0.3) | 5.9 (0.8)* | 3.0 (0.5)* | 2.9 (0.5) |
| 5 | Control[d] | 1.9 (0.2) | 0 (0) | 1.9 (0.2) | 5.1 (0.8) | 1.3 (0.7) | 3.9 (0.2) | 7.0 (0.7) | 2.4 (0.8) | 4.6 (0.5) | 6.9 (0.7) | 2.1 (0.7) | 4.8 (0.5) |
| | Cyclophosphamide[e] | 2.0 (0.3) | 0 (0) | 2.0 (0.3) | 3.3 (0.5)* | 0.4 (0.3) | 2.9 (0.5) | 5.7 (0.6) | 1.5 (0.9) | 4.3 (0.3) | 6.4 (1.2) | 2.0 (0.9) | 4.4 (0.4) |
| | Cyclosporine[f] | 2.0 (0) | 0 (0) | 2.0 (0) | 5.7 (0.7) | 0.6 (0.6) | 5.1 (0.7) | 6.6 (0.6) | 1.5 (0.9) | 5.5 (0.8) | 6.8 (0.4) | 1.5 (0.6) | 5.3 (0.5) |
| | Dexamethasone[g] | 2.3 (0.3) | 0 (0) | 2.3 (0.3) | 4.0 (0.4) | 0.1 (0.1) | 3.9 (0.4) | 4.9 (0.6)* | 0.4 (0.2)* | 4.5 (0.7) | 5.1 (0.6) | 0.9 (0.5) | 4.3 (0.5) |

* Significant difference from control at $P < 0.05$.

[a] 3 ml physiological saline on days −1, 1, 3, 5, and 7 of SRBC inoculation.

[b] 9.1 mg/kg-bw cyclosporine (0.1 ml oral solution) on days −1, 1, 3, 5, and 7 of SRBC inoculation.

[c] 5.5 mg/kg-bw dexamethasone (3 ml injectable) on days −1, 1, 3, 5, and 7 of SRBC inoculation.

[d] 1.6 ml physiological saline on days 0, 1, 3, 5, and 7 of SRBC inoculation.

[e] 20 mg/kg-bw cyclophosphamide (in 1.6 ml physiological saline) on days 0, 1, 3, and 5 of SRBC inoculation.

[f] 9.9 mg/kg-bw cyclosporine (0.1 ml oral solution) on days 0, 1, 3, 5, and 7 of SRBC inoculation.

[g] 5.6 mg/kg-bw dexamethasone (3 ml injectable) on days 0, 1, 3, 5, and 5.0 mg/kg-bw on day 7 of SRBC inoculation.

### Hematology

In Experiment five, the average total white blood cell count for the cyclophosphamide ducks was less than the control value and the total count for the dexamethasone-treated ducks was higher than the control value. These data are not shown here because the standard deviations of the treatment values were large. Lymphocyte percentages ranged from 71% for the control ducks to 52% for the dexamethasone group. Heterophils/eosinophils ranged from 19% in the control ducks to 42% in the dexamethasone-treated ducks. Packed red cell volume ranged between treatments from 35% to 47% and did not appear to be influenced by the treatments.

### DISCUSSION

The in vivo PHA-P skin response is a simple, nonlethal test that measures the amount of swelling following an intradermal injection of PHA-P, a T-cell mitogen. Goto et al. (1978) described the use of this assay with humans as a screening test for cell-mediated immunity. Goto et al. (1978) injected PHA-P into the wattles of chickens and showed that thymectomized chicks have a greatly reduced skin response to PHA-P. Accumulation of lymphocytes and macrophages and, at times, heterophils and basophils was observed at the injection site. McCorkle et al. (1980) examined wattles of chickens injected intradermally with PHA-P and found increased numbers of basophils and eosinophils at 24 hr post injection and also noted changes in the number and types of other cells.

The PHA-P skin test has also been referred to as the cutaneous basophil hypersensitivity response and is related to delayed-type hypersensitivity (DTH). Several studies of DTH with chickens have been conducted as reviewed by Sharma and Tizard (1984). The in vivo DTH response appears to involve a series of immunological steps including mitogen recognition, lymphocyte-macrophage interaction, release of soluble lymphokines, and changes in vascular permeability (Stites, 1984).

For the in vivo skin response to PHA-P, dexamethasone was the only treatment in which a lowered response was observed consistently in the experiments. The action of dexamethasone on the PHA-P response can be explained by the general action of glucocorticoids. Glucocorticoids inhibit migration of T-cell to antigen deposition sites by inhibiting release of lymphokines and by decreasing the number of cells in the marginal zone of the endothelium that participate in inflammatory reactions (Webb and Winkelstein, 1985).

Decreased skin response to PHA-P was expected but not observed in the cyclophosphamide and cyclosporine treated groups. As evidence that the doses were immunosuppressive, three of 10 individuals receiving cyclophosphamide died, 2 to 3 days after the skin test in experiment one. The Animal Health Laboratory of the Wisconsin Department of Agriculture Trade and Consumer Protection (Madison, Wisconsin 53713, USA) diagnosed the cause of death as a staphylococcal infection. For cyclosporine, the doses were similar to those which suppressed serum IgG levels in ducks in Experiment four.

Possible reasons for not seeing decreased PHA-P responses in the cyclophosphamide and cyclosporine treated ducks could include: (1) treatment doses were insufficient to cause suppression, (2) cells responding to mitogen deposited in the skin were not affected by treatment with cyclophosphamide or cyclosporine, or (3) the assay is not sensitive enough to detect impaired function of cells responding to PHA-P.

Conversely, variable pharmacokinetics may have prevented cyclosporine from reaching the cells participating in the skin response. Also, cyclosporine may not have affected the skin response because it suppresses chronic but not acute inflammatory responses (White and Shaw, 1986). The lack of effects by cyclophosphamide may be explained by cyclophosphamide's reported variable effect on cellular immunity (Webb and Winkelstein, 1985). Cyclophosphamide has been shown to exaggerate and prolong dinitrochloroben-

zene contact dermatitis (Maguire and Ettore, 1967) in guinea pigs presumably by inhibiting immune regulation.

The PHA-P skin test requires no prior antigen sensitization as is typical with DTH tests. In addition, this response is not antigen specific and thus does not measure a specific clone of T-cells with antigen specificity. Cyclophosphamide is a cycle specific drug (Webb and Winkelstein, 1985; Winkelstein et al., 1971) and thus its effect on nonantigenic responses may be different from the DTH response. Cyclosporine-A, on the other hand, is specific in the suppression of T-cell dependent responses (White and Shaw, 1986). Since the PHA-P response is T-cell dependent (Goto et al., 1978), it was anticipated that the cyclosporine injected ducks would have suppressed PHA-P responses.

Therefore, the inability of the PHA-P skin test to detect immune suppression in ducks treated with cyclophosphamide or cyclosporine may be due to the characteristics of the immunosuppressive agents, as well as, the immune response measured. Dexamethasone was the only treatment that caused suppression of the skin response and suppression could have been due to dexamethasone's anti-inflammatory action rather than immunosuppression.

The in vivo immune response to SRBC is determined by quantifying serum antibody titers. This assay is an inexpensive, simple, and nonlethal measure of immune function. Proper function of all three cell types of the immune system (i.e., T-cells, B-cells and the macrophage) is essential for a normal antibody response. In addition, turnover of plasma immunoglobulin is also reflected in the results of this assay.

In general, cyclophosphamide had no effect on the antibody response of the ducks. Cyclophosphamide is cytotoxic in a dose-related manner to cells that are capable of replication (i.e., stimulated B-cells, Ficken and Barnes, 1988). Antibody is produced by plasma cells which are not capable of replication. Since preinoculation titers contain antibody to SRBC in most avian species, plasma cells capable of an-

tibody synthesis without replication could mask the effects of cyclophosphamide (Miller and Cole, 1967). Therefore, cyclophosphamide does not appear to be cytotoxic for plasma cells (Miller and Cole, 1967; Webb and Winkelstein, 1985). In addition, cyclophosphamide has not been shown to affect the function of remaining viable immune cells (Ficken and Barnes, 1988), although reduced antibody levels in neonatally treated pekin ducklings has been observed (Hashimoto and Sugimura, 1976).

The results of experiment four suggest that cyclosporine reduces IgG levels but not IgM and total antibody to SRBC. Cyclosporine may preferentially bind to T-helper cells and inhibit expression of interleukin-2 (IL-2) receptors and may inhibit the secretion or production of IL-2 (White and Shaw, 1986). Isakson et al. (1982) suggested that IgG production requires help from T-helper cells. Therefore, cyclosporine may have prevented IgG production by interfering with T-helper cell function. However, results from both of the experiments reported here plus preliminary experiments not reported (Schrank, 1989), in which lower doses were administered, show that the effect of cyclosporine on antibody levels may be variable.

A possible reason for the variable effect of cyclosporine may be that intramuscular injection of cyclosporine does not always provide reproducible pharmacokinetics (Bach and Strom, 1985). Treatment in experiment four began on the day prior to antigen inoculation and on the day of antigen inoculation in experiment five. Earlier injection in experiment four may have allowed greater distribution of the cyclosporine to cells affecting antibody production compared to experiment five.

Corticosteroids have been shown to reduce antibody responses by inhibiting synthesis of IgG or by increasing the catabolism of IgG. Reduced antibody production is thought to be through reduced IL-1 secretion or suppressed IL-2 synthesis by T-helper cells (Webb and Winkelstein, 1985). Corticosteroids are considered po-

tent suppressors of humoral immunity in cortico-sensitive species by causing lysis of lymphocytes (Bach, 1975). Dexamethasone consistently depressed peak total and IgG antibody levels in experiments four and five. Preliminary experiments (Schrank, 1989) using lower doses did not produce differences in antibody production to red blood cells.

Results of these experiments show that antibody levels produced in response to SRBC potentially could be used as an in vivo tool to evaluate immune competence of mallard ducks in field experiments using dexamethasone and cyclosporine as positive treatment controls along with normal controls. Further testing should be conducted to investigate if these altered responses are reflective of actual disease resistance.

Results of these experiments suggest that the mode of action of the immune suppressant used is an important factor in the ability of immune function tests to detect immune modulation. This evidence reinforces the suggestion by others (Dean et al., 1986) that a panel of immune function tests must be used to examine possible immune modulation. It may be especially important to use a panel of immune function tests when evaluating possible effects of environmental contaminants that could probably have complex and diverse effects on the immune system of waterfowl.

## ACKNOWLEDGMENTS

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