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THE EFFECT OF DIETARY DEOXYNIVALENOL ON SERUM IMMUNOGLOBULIN A IN THE MOUSE

By

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ABSTRACT

THE EFFECT OF DIETARY DEOXYNIVALENOL ON SERUM IMMUNOGLOBULIN A IN THE MOUSE

By

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Fusarium mycotoxins are commonly found in cereal grains and when consumed are frequently associated with serious animal health problems. Recently the consumption of mycotoxins has been associated with immunosuppression. The objective of this research was to examine the effect of the trichothecene mycotoxin deoxynivalenol (DON) on total and specific immunoglobulin in the mouse. Exposure to 25 ppm dietary deoxynivalenol caused a decrease in serum immunoglobulin G (IgG) and immunoglobulin M (IgM) but increased immunoglobulin A (IgA) up to 17-fold after 24 weeks of feeding. Serum from mice fed deoxynivalenol and challenged with cholera toxin showed decreased cholera toxin-specific serum IgG but not IgA. Casein specific serum IgA from these mice was increased whereas casein-specific serum IgG was decreased. Isolated splenocytes from mice fed deoxynivalenol exhibited increased IgA in mitogen and non-mitogen stimulated cultures. These results suggest that dietary exposure to deoxynivalenol alters regulation of IgA production.

This thesis is dedicated to the memory of my father.

Eugene Frank Moorman

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Introduction

Mycotoxins are secondary metabolites produced by certain fungi (molds) that when consumed can cause significant health effects in humans and animals. There have been many reported outbreaks of mycotoxicoses due to the consumption of mycotoxins in humans dating back to the occurence of ergotism or "holy fire" during the middle ages (Davis and Diener, 1987). Humans consuming bread prepared from rye infected with Claviceps purpurea were affected with either convulsive or gangrenous ergotism (Floss and Anderson, 1980). The patient experienced violent, burning pains in extremities (hence the Fire of St. Anthony). The part gradually became numb, and gangrenous.

Two other historic outbreaks of mycotoxins pointed out their importance to human and animal health. One came about in England in the 1960's with the death of more than 100,000 turkey poults in what is called the Turkey-x disease (Davis and Diener, 1987; CAST Report, 1979). The causative agent was found to be aflatoxin produced by Aspergillus flavus in the peanut meal fraction of the feed. More recently it has been shown that of the aflatoxins present (B_1, B_2, G_1, G_2), aflatoxin B_1 is one of the most carcinogenic naturally occuring substances known (CAST Report, 1979). Alimentary Toxic Aleukia (ATA) was caused by the consumption of Fusarium

contaminated grain resulting in severe outbreaks between 1930 and 1950. A 1932 outbreak of ATA in one district of Russia resulted in mortality of 62% of those afflicted (Davis and Diener, 1987). Consumption of the spoiled grains resulted in severe bone marrow suppression, anaemia, bleeding, and immune suppression with the infectious complications leading to death after several weeks (Bunner et al., 1985). It was later determined that the tricothecene T-2 was the principal toxin involved.

The focus of this thesis research is on one chronic effect of trichothecene mycotoxins, namely, immunosuppression. The trichothecene mycotoxins are a specific group of fungal metabolites based on their common trichothecane structure. The skeleton structure includes a six-membered oxygen containing ring, an epoxide group in the 12, 13 position, and an olefinic bond in the 9,10 position. The chemical structure of the trichothecene mycotoxin deoxynivalenol is presented in Fig 1. These naturally occuring compounds are produced by various genera of fungi such as Tricothecium, Trichoderma, Myrothecium, Cephalosporium, Fusarium, Stachybotrys, Verticimonosporium, and Cylindocarpon. The 45 known trichothecenes can be categorized into groups A,B,C, or D according to their chemical characters and the particular producing fungi (Ueno, 1983). Since the first isolation of a trichothecene mycotoxin in 1949 these compounds have postulated to be etiological agents in a variety of human and animal disease outbreaks. Japan has been especially afflicted with this

Figure 1. The chemical structure of deoxynivalenol. The epoxide structure is essential for toxic action (Ueno, 1983).

problem dating back to 1890 with what is called the Red Mold Disease. The Fusarium genus is responsible for these outbreaks when it grows in wheat, barley, oats, rye, rice and other crops primarily during heavy raining seasons. When humans or farm animals consume these commodities, nausea, vomiting, diarrhea, feed refusal, congestion or hemorrhage in the lung, adrenals, intestine, uterus, vagina and brain, and destruction of the bone marrow are the characteristic symptoms (Ueno, 1983).

Deoxynivalenol (DON or vomitoxin) is a trichothecene mycotoxin produced by Fusarium graminearum and is a common contaminant of cereal grains worldwide (Mirocha et al., 1976). Infection of corn and cereal grains by Fusarium graminearum and concomitant DON production occur most frequently in years that are particularly cool and wet at times of harvest (Cote et al., 1984). DON produced in feedstuffs has been shown to survive processing involved in manufacture of the commodity (Greenhalgh et al., 1984) to the extent that in an FDA market survey, 60% of breakfast cereal tested contained DON at an average level of contamination of approximately 100 ppb (Trucksess et al., 1986).

Farm animals that consume corn and cereal grains containing DON develop various health problems such as feed refusal, reduced weight gain, reproductive problems, diarrhea, emesis, and death. Swine are especially susceptible to the effects of the toxin giving DON the name vomitoxin due to its emetic activity (Forsyth et al., 1977). In 1982, the Centralia Diagnostic Laboratory and the

laboratory at the University of Illinois found that 80% of the feed samples tested that were suspected of causing or contributing to animal health problems contained DON at a concentration ranging from 0.1 to 41.6 ppm (mean 3.1 ppm) (Cote et al., 1984). In 1982, 33 samples of wheat from Kansas and Nebraska were analyzed for the mycotoxins DON, T-2 toxin, zearalenone, and aflatoxin. DON was identified in 31 of 33 samples (Hagler et al., 1984).

Trichothecenes inhibit protein and DNA synthesis and are therefore generally considered to have immunosuppressive properties (Miller and Atkinson, 1987). Pier et al. (1980) noted that subacute mycotoxin exposure can lead to impairment of immunogenesis and native mechanisms of resistance thus leading to secondary mycotoxin diseases. Both humoral and cellular immunity can be affected by small quantities of mycotoxins which can eventually lead to profound alterations in the defense mechanisms of the body against infection. Experimentally, acute doses of DON have resulted in extensive necrosis of the gastro-intestinal tract, bone marrow and lymphoid tissues, and focal lesions in kidney and cardiac tissue (Forsell et al. 1987). Acute and chronic toxicities of trichothecenes include depletion of lymphoid tissues, which strongly suggest that the trichothecenes can alter immune responses (Ueno 1983). When Listeria monocytogenes infection is used as a measure of immune resistance, dietary DON causes a decreased time-to-death interval of mice (Tryphonas et al., 1986). Using a similar model, Pestka et al., (1986) demonstrated that 25 ppm DON induced higher

splenic Listeria monocytogenes counts than the ad lib control indicating a decreased ability to fight disease.

The specific effects of trichothecenes on the immune system have been studied by examining the cellular responses of lymphocytes to these toxins. Lymphocyte blastogenesis is one valid in vitro model for determining the effect of environmental contaminants on immunocompetent cells (Forsell and Pestka, 1985). Lafarge-Frayssinet et al. (1979) observed a decrease in mitogen-stimulated blastogenesis of B and T cells after experimental treatment of mice with Fusarium extracts containing T-2 toxin and diacetoxyscirpenol. Mitogen-induced blastogenesis in human peripheral blood lymphocytes is inhibited by 50% with approximately 1.5 ng/ml T-2 toxin (Cooray, 1984; Forsell et al., 1985; Tomar et al., 1988) Robbana-Barnat et al. (1988) showed that 131 ng/ml DON decreased murine splenocyte blastogenesis by 50%. Assuming equivalent sensitivity of human and murine lymphocytes, these experiments indicate the greater toxicity of T-2 toxin and demonstrates the extreme potency of these toxins.

There is some conflicting evidence for stimulation or increased blastogenesis in lymphocytes treated with low concentrations of trichothecene mycotoxins. Lafarge-Frayssinet et al. (1979) observed an increase in blastogenesis in lymphocytes of mice treated with low doses of Fusarium extracts containing T-2 toxin and diacetoxyscirpenol. Forsell et al. (1985) also observed a slight stimulation of lymphoblastogenesis with low (5×10^{-4} to 5×10^{-3} ng/ml) T-2 toxin concentrations. Robbana-Barnat

et al. (1988) could not duplicate these results with low in vitro doses of DON.

Robbana-Barnat et al. (1988) examined the effects of DON, the most commonly occuring trichothecene, on murine lymphoid organs and specifically on humoral immunity. Orally administered DON causes significant reduction of thymus and splenic weights and the authors observed that sensitivity to DON was greater with lymphocytes than fibroblastic cells. Sheep red blood cells (sRBC) are T-cell dependent for immune response and are commonly used to study humoral immunity. Oral DON has been shown to lower serum antibody responses to sheep red blood cells (Robbana-Barnat et al., 1988), specifically decreasing serum IgM (Tryphonas et al., 1984). Robbana-Barnat et al. (1988) speculated that DON could directly impair antibody formation to sheep red blood cells by adversely affecting the functional integrity of a subset of the T-lymphocyte, possibly the helper T-cell. This conclusion is supported by DON-induced decreased thymus weights (Tryphonas et al., 1984; Robbana-Barnat et al., 1988; Pestka et al, 1986). Forsell et al. (1986) also observed a dose-dependent decrease in serum IgM upon dietary DON exposure but, in contrast a dose-dependent increase in serum IgA. The IgA concentration was maximal at 10 ppm DON decreasing at the 25 ppm level. Consistent with this was the observation of Cooray and Lindahl-Kiessling (1988) that there was a dose-dependent increase in the number of spontaneous antibody secreting cells from the spleen of mice treated with T-2 toxin. Based on these and previous results (Otakawa et

al. (1979)), the authors speculated that the increase in antibody producing cells could be due to the selective destruction of T suppressor cells. In conflict to this hypothesis, Tomar et al. (1988) demonstrated that T-2 toxin did not induce the generation of suppressor cells by the T cell mitogen concanavalin A.

IgA serves a very important role at mucosal surfaces where it binds potentially infectious agents and prevents their absorption. The dose-dependent increase in serum IgA noted by Forsell et al., (1986) at DON levels found frequently in agricultural commodities was unexpected. The objective of this project was to further characterize the effect of dietary DON on serum IgA. Particular attention was placed on evaluating the dose effects, and determining the kinetics at the optimal dose. The effect of dietary DON on mitogen induced splenic lymphocyte immunoglobulin production was evaluated to determine if the spleen was involved in the elevation of serum IgA. It is anticipated that these studies will result in a model that can be used to study mechanisms by which dietary DON alters serum immunoglobulin levels.

Materials and Methods

General Experimental Design. The research in this thesis was designed to develop a model to study the effects of dietary DON on serum and mucosal immunoglobulin production. The mice were fed diet that contained DON and the serum and mucosal compartments were analyzed for total and antigen specific immunoglobulin. Based on the results of these feeding studies, the model was extended to evaluating the immunoglobulin response of mitogen-stimulated splenocytes from mice.

Deoxynivalenol. Deoxynivalenol was produced in Fusarium graminearum R6576 cultures and purified by water-saturated silica gel chromatography (Witt et al. 1985)

Diet. Mice were fed powdered AIN-76A semi-purified diet (ICN Nutritional Biochemicals, Cleveland, OH) to minimize non-specific immune effects caused by nutrient variability. Purified DON was incorporated into the diet as described by Forsell et al. (1986). Briefly, DON was first dissolved in ethanol and added to the powdered diet to yield a 1000 ppm concentrate. After being mechanically mixed fo 10 minutes and dried overnight under a fume hood, the concentrate was mixed with diet to obtain the desired mycotoxin levels. During experiments all diets were stored in the dark at 10^{o}C.

Animals B6C3F_1, weanling female mice purchased from

Harlan Sprague Dawley, Inc. were housed individually in protected environment cages. Each cage unit included a transparent poly carbonate body with filter cover, stainless-steel wire lid, and a raised cage floor over treated hardwood chips. Mice were given distilled water ad libitum.

Feeding Protocol. Experiments consisted of a DON treatment group and control groups. Treatment groups received fresh DON diet every 3 days with the control group receiving control diet every 3 days. Experiments with restricted control groups received control feed at a dosage equivalent to the previous 3 day mean intake for the DON treatment group (Pestka et al., 1987).

Sample Collection. Blood was obtained from the retro-bulbar plexus of ether-anesthetized mice by using non-heparinized capillary tubes. After centrifugation of samples, serum was removed and stored at -20^{o}C until assay.

Saliva was obtained from mice after intraperitoneal (i.p.) injection with 1 mg pilocarpine (Sigma Chemical) in 0.2 ml water (Challacombe and Tomasi, 1980). After 2 minutes saliva was collected in small glass tube, diluted 1:1 with 1:1000 Thimersol^{R} (Sigma Chemical) and stored at -20^{o}C until assay.

Intestinal contents were collected by the procedure of Snider and Underdown (1986) with minor modifications. In short, mice were deprived of food for 2-4 hours prior to intestine removal. After determining body weight, mice were sacrificed in a CO_2 chamber. The mouse abdomen was washed with 70% ethanol, and the whole small intestine was removed

below the stomach and ligated within 4 cm of the end of the ileum. Ice cold trypsin inhibitor solution (1.5 ml) was injected into the duodenal end and the intestine was massaged for 1 minute. Trypsin inhibitor solution was composed of 0.1 M phosphate buffered saline (PBS, pH 7.2), 5mM ethylenediaminetetraacetic acid trisodium salt (EDTA), 2mM phenylmethysulfonylfluoride (PMSF) (Sigma Chemical) and 0.05 U aprotinin (trypsin inhibitor) (Sigma Chemical). Contents were drained into a sterile petri dish and transfered to a 15 ml conical tube. Supernatants were collected after centrifugation at 800 x g for 10 minutes at 4^{o}C and then frozen at -20^{o}C until assayed.

Immunoglobulin quantitation. Total serum immunoglobulin was quantitated by radial immunodiffusion (RID) according to the method of Luster et al. (1982). RID-agarose gel for 1 liter consisted of 6.98 g sodium barbital, 6.0 g sodium chloride and 0.1 g Thimersol^{R}. After adjusting pH to 7.6 with concentrated (38%) HCL, 10 g agarose (Bethesda Research Laboratories, Gaithersburg MD) was added and dispensed into 9 ml tubes. RID plates (Miles Scientific, Naperville IL) were prepared by adding goat antimouse immunoglobulin (Cooper Biomedical, West Chester PA) to 9 ml of tempered RID agarose gel (62^{o}C) and pouring onto RID plates. Optimized dilutions were as follows: goat anti-mouse IgA (alpha chain specific), 1:60; IgM (mu chain specific), 1:70; and IgG (gamma chain specific), 1:50. After allowing plates to solidify for 1 hr at 25^{o}C, 3 mm holes were cut with a template (Miles Scientific) and 10 ul of serum sample or mouse reference

serum (ICN ImmunoBiologicals, Lisle, IL) were added. IgA serum samples were diluted 1:1 in (0.85%) saline, IgM samples 1:3 and IgG samples 1:6. RID plates were incubated in a humid chamber for 48 hours (IgG and IgM plates for 24 hours) at 4°C. The resultant precipitin bands were measured with a vernier caliper and immunoglobulin concentrations were determined based on the precipitin bands obtained with reference serum (Miles Scientific, Naperville IL).

Salivary IgA was estimated by the enzyme linked immunosorbent assay (ELISA). For this assay, goat-antimouse IgA (alpha chain specific) was diluted to 10 ug/ml in 0.1 M bicarbonate buffer (pH 9.6) and 100 ul incubated in Immulon 2 Removawell Microtiter Strips (Dynatech Laboratories, Chantilly, VA) overnight at 4°C. Coated plates were then washed 3 times with 0.1 M phosphate buffered saline (pH 7.2; PBS) containing 0.2% Tween 20 (PBS-Tween). To reduce non-specific protein binding, 0.3 ml 1% (w/v) bovine serum albumin (BSA) in PBS were added to each well. The plates were incubated at 37°C for 30 min. and then washed 4 times with PBS-Tween. One hundred microliter aliquots of saliva or immunoglobulin reference serum both diluted in 1% BSA-PBS were added to appropriate wells and incubated for 1 hr at 37°C. After washing 5 times with PBS-Tween, 0.1 ml goat antimouse IgA peroxidase (alpha chain specific, Cooper Biomedical) diluted 1:500 in 1% BSA-PBS was added to each well. After 30 minutes the plate was washed 8 times. Bound peroxidase was determined by incubating 100 ul ABTS-H_2O_2 substrate in each well for 5-30 minutes, and the reaction was

terminated with 100 ul citric acid stopping solution (Pestka et al, 1980). Absorbance at 405 nm was determined on an EIA Minireader 2 (Dynatech Product, Alexandria VA). IgA was quantitated by extrapolation from reference curves.

Intestinal IgA was quantitated by the same ELISA protocol with a few modifications. The sample and reference serum volume added to the wells was 50 ul with the sample diluted 1:2. Goat antimouse IgA peroxidase was diluted 1:3000 in 1% BSA-PBS with a 50 ul volume added to the well.

Cholera toxin and casein specific immunoglobulin quantitation. One (1) mg of cholera toxin from Vibrio cholerae (Sigma Chemical, ST. Louis MO) was dissolved in 1.0 ml sterile distilled water. After dilution and filter sterilization, 0.2 ml was gavaged orally with 20 gauge intubation needle at a dose of 10 ug/mouse. Cholera toxin and casein antibody titers were measured by ELISA. In this method, 50 ul of 10 ug/ml cholera toxin, casein, or goat antimouse immunoglobulin (alpha or gamma chain specific) diluted in 0.1 M bicarbonate buffer (pH 9.6) were adsorbed onto microtiter strips (Dynatech laboratories, Chantilly, VA) by incubation overnight at 4^{o}C. Following incubation plates were washed 3 times with PBS-Tween and then blocked with 1% ovalbumin in PBS (OA-PBS) for 1 hour at 37^{o}C to prevent non-specific binding. After washing 4 times, 50 ul of serum sample diluted 1:10 in 0.1 M PBS were added to the wells coated with cholera toxin or casein. At this same time, 50 ul of serially diluted reference serum were added to anti-immunoglobulin wells which were flanked by the cholera toxin

and casein wells. These plates were incubated 1 hour at 37°C. After washing 5 times with PBS-Tween, 50 ul of goat anti-mouse immunoglobulin peroxidase conjugate diluted in 1% OA-PBS (IgA : 1:500; IgG : 1:750) were added to all wells of plate and incubated 30 minutes at 37°C. The plates were then washed 6 times. Bound peroxidase was determined by incubating 100 ul ABTS-H_2O_2 substrate in each well for 5-30 minutes, and the reaction was terminated with 100 ul stopping solution (Pestka et al, 1980). Absorbance at 405 nm was determined on an EIA Minireader 2 (Dynatech Product, Alexandria, VA). Immunoglobulin was quantitated by extrapolation from standard reference curves.

In vitro immunoglobulin production. Single cell spleen suspensions were prepared after passing the spleen through an 85 mesh screen tissue grinder (Thomas Scientific, Swedesboro NJ) into a petri dish. The screen was then washed with RPMI-1640 (Sigma Chemical Company, St. Louis MO) supplemented with 20% fetal calf serum (FCS) (Gibco Laboratories), 1% sodium pyruvate (Sigma Chemical), 2 mM L-Glutamine (Sigma Chemical), 5×10^{-5} M 2-mercaptoethanol (Sigma Chemical), 1% non-essential amino acids (Sigma Chemical), and Penicillin (100 U/ml)-Streptomycin (100 microgram/ml) (Gibco Laboratories). Cells were collected and placed on ice for 10 minutes to let debris settle. The supernatant was then washed twice by centrifugation at 450 x g for 8 minutes. Viable cells were enumerated with a hemocytometer by trypan blue exclusion. Spleen cell concentrations were adjusted to 2.5×10^6 cells/ml. The cell suspension (200 ul) was transferred to

wells of flat bottomed 96-well tissue culture plates (Costar, Cambridge MA). Mitogen (10 ul) was added to all wells except the spontaneous control which received an equivalent volume of sterile distilled water. The final concentration of mitogen used per ml of culture was as follows: LPS, 20 ug/ml; Con A, 10 ug/ml; and Con A + LPS, 10 and 20 ug/ml, respectively. Cultures were incubated for 7 days in a 5% CO_2 incubator.

In vitro production of IgA, IgG, and IgM was measured by ELISA. For this assay, 100 ul of 10 ug/ml goat antimouse immunoglobulin (alpha, gamma, mu specific) diluted in 0.1 M bicarbonate buffer (pH 9.6) were coated onto microtiter strips overnight at 4°C. Following incubation, coated plates were washed 3 times with PBS-Tween and then blocked with 0.3 ml 1% OA-PBS (Sigma Chemical) for 30 minutes at 37°C. After washing 4 times with PBS-tween, 50 ul of supernatant or reference serum both diluted in 20% FCS-RPMI-1640 were incubated for 1 hour at 37°C. IgA supernatants were diluted 1:2 with IgG and IgM supernatants diluted 1:4. After washing 5 times, 50 ul of goat anti-mouse IgA peroxidase conjugate (diluted 1:500 in 20% FCS-RPMI-1640) was added for 30 minutes at 37°C. Goat antimouse IgG and IgM peroxidase diluted 1:750 in 20% FCS-RPMI-1640. After washing 8 times, total immunoglobulins were determined as described above.

RESULTS

Serum immunoglobulin profile in mice following dietary deoxynivalenol exposure. Exposure to dietary DON for 8 weeks has been previously shown to cause a dose-dependent decrease in serum IgM and a dose-dependent increase in serum IgA (Forsell et al., 1986). The minimum DON concentration to increase serum IgA was 2 ppm, maximum IgA at 10 ppm, and decreasing at 25 ppm DON. The first objective of this project was to further characterize the effect of dietary DON on serum immunoglobulin levels. Particular attention was placed on replicating the dose effect, determining the kinetics at the optimal dose, and evaluating the possible effect DON-induced feed refusal has on serum immunoglobulin. To measure this latter effect, a "restricted" control group which received clean AIN-76A diet at levels equivalent to the mean intake of dietary DON for the treatment group was included.

The effect of 2 and 10 ppm dietary DON on serum IgA over an 8 week period is presented in Table 1 (Exp. 1). Serum IgA in the 10 ppm DON group was significantly increased relative to the restricted and ad lib control groups at weeks 2 and 4 ($p<0.05$). This group was elevated when compared to the restricted and ad lib control groups at weeks 6 and 8 but the difference was not significant due to the large amount of variation. IgA was not significantly affected in the 2 ppm DON group.

Table 1. Serum IgA concentrations in mice following dietary exposure to 2 and 10 ppm DON.[a]

Treatment	IgA Log_{10}(ug/ml)[b] wk 2	wk 4	wk 6	wk 8
2 ppm DON	2.63 ± 0.23 (93)	2.66 ± 0.09 (102)	2.80 ± 0.11 (118)	2.88 ± 0.14 (107)
2 Restricted	2.66 ± 0.17 (96)	2.60 ± 0.10 (91)	2.74 ± 0.13 (103)	2.80 ± 0.07 (85)
10 ppm DON	2.77 ± 0.09[c,d] (122)	2.76 ± 0.20[c,e] (141)	2.85 ± 0.13 (130)	2.98 ± 0.20 (141)
10 Restricted	2.63 ± 0.09[d] (90)	2.62 ± 0.06[e] (94)	2.76 ± 0.10 (106)	2.88 ± 0.22 (114)
ad lib control	2.68 ± 0.09	2.64 ± 0.06	2.73 ± 0.12	2.85 ± 0.10

[a] $B6C3F_1$ treatment mice were fed 2 or 10 ppm DON in AIN-76A diet. (Exp 1)

[b] Data shown are geometric mean ± standard deviation for groups of 22 controls and 10 treated mice. Numbers in parentheses are (ratio of means of treatment to ad lib) X 100. Difference between treatment groups and ad lib control determined by Dunnett's t test. Difference between treatment groups and their restricted controls determined by the improved Bonferroni t test.

[c] Numbers with a superscript c are significantly different than the ad lib control ($p<0.05$).

[d-e] Groups with identical letters are significantly different.

A second experiment (Exp 2) was initiated to examine serum IgA, IgG, and IgM at 10 ppm DON using a larger number of animals (n=16) than the previous experiment (n=10). The 10 ppm restricted control was omitted from this experiment since IgA in the restricted group was not elevated in the prior experiment suggesting that decreased feed intake did not effect serum IgA concentrations at 10 ppm dietary DON. Table 2 presents the serum immunoglobulin concentrations over 8 wks following 10 ppm DON exposure (Exp 2). Serum IgA was significantly elevated in the 10 ppm DON group at weeks 4 and 8 ($p<0.01$) and at week 6($p<0.05$) while no effect was seen on serum IgM. Serum IgG was significantly decreased ($p<0.05$) in the 10 ppm DON group at week 8.

A third study (Exp 4) was initiated to determine the effects of higher DON levels (25 and 50 ppm) on serum immunoglobulin concentration over time (Table 3). Serum IgA was significantly elevated relative to its restricted control from weeks 2 through 12 ($p<0.05$) and significantly elevated relative to the ad lib control at weeks 10, and 12 ($p<0.05$). Figure 2 presents the serum immunoglobulin concentrations after 12 weeks of 25 ppm dietary DON.

Mortality was not observed in mice fed 25 ppm whereas all animals recieving 50 ppm died before wk 4 of dietary DON feeding. This represents a toxic level of DON.

Serum IgM in 25 ppm DON treatment mice was significantly decreased relative to its restricted and ad lib controls from wk 4 to wk 12 ($p<0.05$). Serum IgG at 25 ppm DON was significantly decreased when compared to the

Table 2. Serum immunoglobulin concentrations in mice following dietary exposure to 10 ppm DON.[a]

	Immunoglobulin concentration Log_{10}(ug/ml)[b]				
Treatment	wk 0	wk 2	wk 4	wk 6	wk 8
	IgA				
10 ppm DON	2.87 ± 0.51 (138)	2.76 ± 0.12 (112)	2.86 ± 0.10[d] (126)	2.77 ± 0.10[c] (115)	3.02 ± 0.19[d] (145)
ad lib control	2.73 ± 0.08	2.71 ± 0.08	2.76 ± 0.03	2.71 ± 0.04	2.86 ± 0.09
	IgG				
10 ppm DON	3.70 ± 0.24 (126)	3.66 ± 0.18 (107)	3.59 ± 0.18 (91)	3.62 ± 0.16 (81)	3.65 ± 0.12[c] (81)
ad lib control	3.60 ± 0.11	3.63 ± 0.10	3.63 ± 0.11	3.71 ± 0.11	3.74 ± 0.12
	IgM				
10 ppm DON	2.30 ± 0.15 (120)	2.26 ± 0.10 (89)	2.32 ± 0.12 (95)	2.36 ± 0.10 (93)	2.39 ± 0.13 (138)
ad lib control	2.22 ± 0.11	2.31 ± 0.10	2.34 ± 0.08	2.39 ± 0.10	2.25 ± 0.59

[a] Mice were fed 10 ppm DON in AIN-76A diet for 8 weeks. (Exp 2)

[b] Data shown are geometric mean ± standard deviation for groups of 16 mice. Difference between 10 ppm DON and ad lib control groups determined by Dunnett's t test. Values in parantheses are percent change relative to the ad lib control.

[c] Significantly different than ad lib control ($p<0.05$)

[d] Significantly different than ad lib control ($p<0.01$)

Table 3. Serum immunoglobulin concentrations in mice following dietary DON exposure.[a]

Treatment	Immunoglobulin concentration Log_{10}(ug/ml)[b]						
	wk 0	wk 2	wk 4	wk 6	wk 8	wk 10	wk 12
	IgA						
25 ppm DON	2.83 ± 0.15	2.89 ± 0.13[c]	2.86 ± 0.15[c]	2.86 ± 0.22[c]	2.98 ± 0.25[c]	3.19 ± 0.24[c,d]	3.40 ± 0.14[c,d]
25 Restricted	2.75 ± 0.09	2.69 ± 0.13[d]	2.67 ± 0.15	2.66 ± 0.08	2.63 ± 0.12	2.69 ± 0.16	2.71 ± 0.13
ad lib control	2.86 ± 0.12	2.75 ± 0.13	2.74 ± 0.10	2.72 ± 0.08	2.76 ± 0.11	2.70 ± 0.15	2.77 ± 0.15
	IgG						
25 ppm DON	3.61 ± 0.17	—	3.33 ± 0.11[c,d]	—	3.42 ± 0.12[c,d]	—	3.34 ± 0.17[c,d]
25 Restricted	3.65 ± 0.08	—	3.45 ± 0.10[d]	—	3.60 ± 0.13[d]	—	3.52 ± 0.11[d]
ad lib control	3.68 ± 0.11	—	3.67 ± 0.11	—	3.84 ± 0.16	—	3.83 ± 0.18
	IgM						
25 ppm DON	2.23 ± 0.17	—	2.17 ± 0.14[c,d]	—	2.17 ± 0.15[c,d]	—	2.18 ± 0.17[c,d]
25 Restricted	2.18 ± 0.17	—	2.37 ± 0.16	—	2.44 ± 0.13	—	2.43 ± 0.21
ad lib control	2.12 ± 0.11	—	2.50 ± 0.20	—	2.48 ± 0.09	—	2.58 ± 0.15

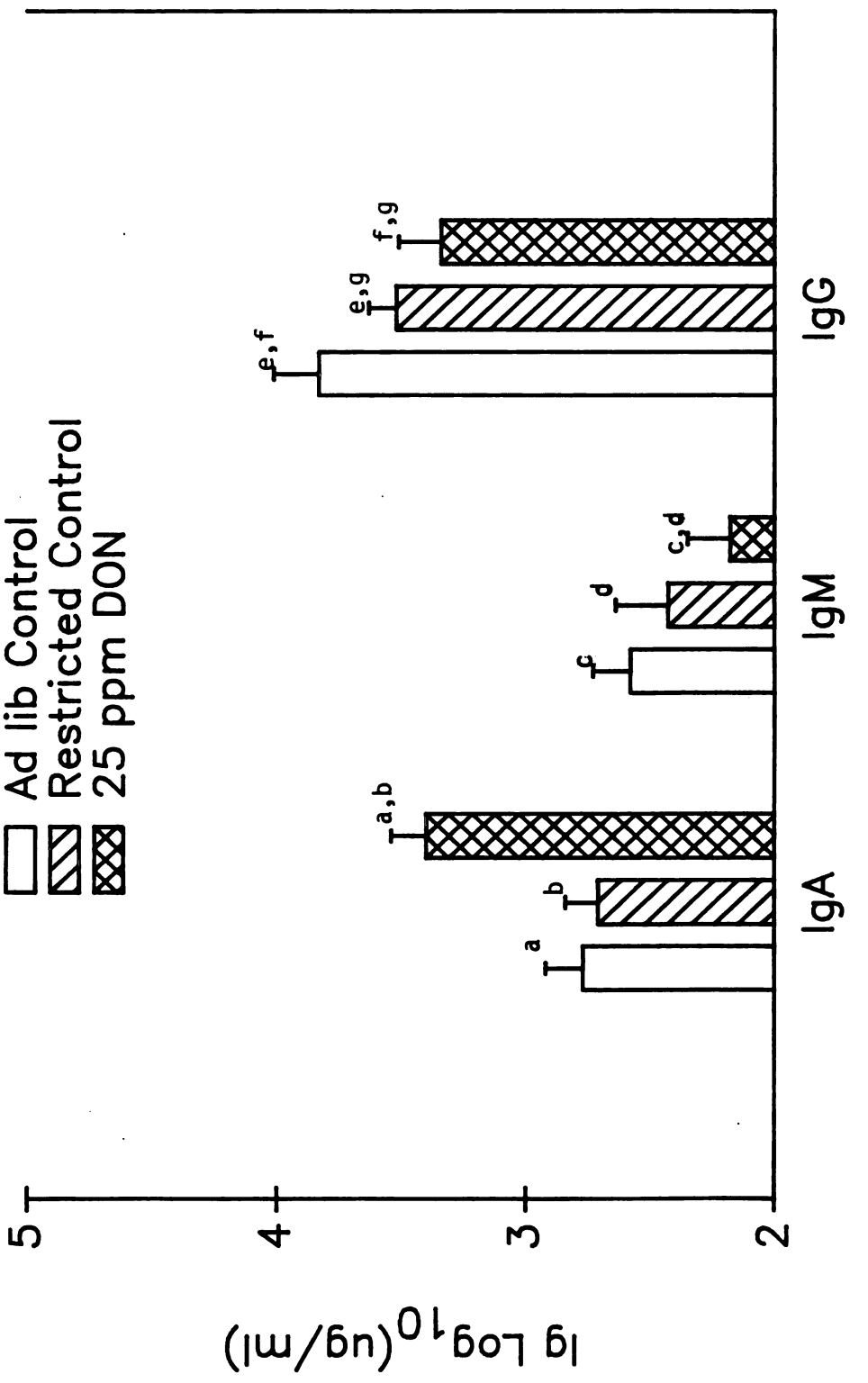
[a] Mice were fed 25 ppm DON in AIN-76A diet for twelve weeks. (Exp 4)

[b] Data shown are geometric means ± standard deviation for groups of 6 control and 8 treated and restricted control mice. Differences between treatment groups and ad lib control determined by Dunnett's t test. Differences between treatment groups and their restricted controls determined by the improved Bonferroni t test.

[c] Significantly different than restricted control within immunoglobulin ($p<0.05$).

[d] Significantly different than ad lib control within immunoglobulin ($p<0.05$)

Figure 2. Serum immunoglobulin isotype profile following dietary DON exposure. Mice were fed 25 ppm DON in AIN-76A diet for 12 weeks. Data shown are geometric means ± S.D. for groups of 6 ad lib control and 8 treated and restricted control mice. Difference between treatment and ad lib control determined by Dunnett's t test. Difference between treatment and restricted or restricted and ad lib control determined by the improved Bonferroni t test. Treatment and control groups with identical letters are significantly different.


Ad lib Control
Restricted Control
25 ppm DON
lg Log10(ug/ml)
5
4
3
2
IgA
IgM
IgG
a
b
a,b
c
d
c,d
e,f
e,g
f,g

restricted and ad lib controls from wk 4 to wk 12 ($p<0.05$). Serum IgG was also significantly decreased ($p<0.01$) in the 25 restricted group when it is compared to the ad lib control from wk 4 to wk 12. This indicates that depressed IgG and IgM levels could result from decreased feed intake whereas the elevated IgA was a specific DON-related effect.

The potent effect of 25 ppm DON on serum immunoglobulin was replicated in a later experiment (Table 4). This experiment (exp 5) contained a greater number of replicates for statistical power than the previous 25 ppm experiment. These animals also served as sources of spleens for the *in vitro* experiments which will be discussed later. Here the 25 ppm DON group was significantly elevated ($p<0.01$) when compared to the restricted and ad lib control groups at weeks 4 through 12. The DON treatment group after 12 weeks had a serum IgA concentration that was elevated over 4-fold relative to the ad lib control.

A fourth study (Exp 6) was initiated that measured the long term effects of exposure to 25 ppm DON. Table 8 presents the serum IgA concentrations following 6 months of 25 ppm DON exposure. The 25 ppm DON and ad lib control groups were present as controls for the cholera toxin experiments which will be discussed in the antigen specific section. The 25 ppm DON group was significantly elevated ($p<0.01$) from week 4 to week 24. Serum IgA concentrations steadily increased over time (Fig 3). After 24 weeks the 25 ppm DON group was elevated over 17-fold relative to the ad lib control.

Table 4. Serum IgA concentration in mice following dietary exposure to 25 ppm DON[a]

	IgA Log_{10}(ug/ml)[b]			
Treatment	wk 0	wk 4	wk 8	wk 12
25 ppm DON	2.80 ± 0.15	3.00 ± 0.13[c,d]	3.22 ± 0.22[c,d]	3.38 ± 0.25[c,d]
25 Restricted	2.75 ± 0.07	2.72 ± 0.08	2.69 ± 0.04	2.89 ± 0.09
ad lib control	2.78 ± 0.11	2.76 ± 0.09	2.68 ± 0.08	2.77 ± 0.09

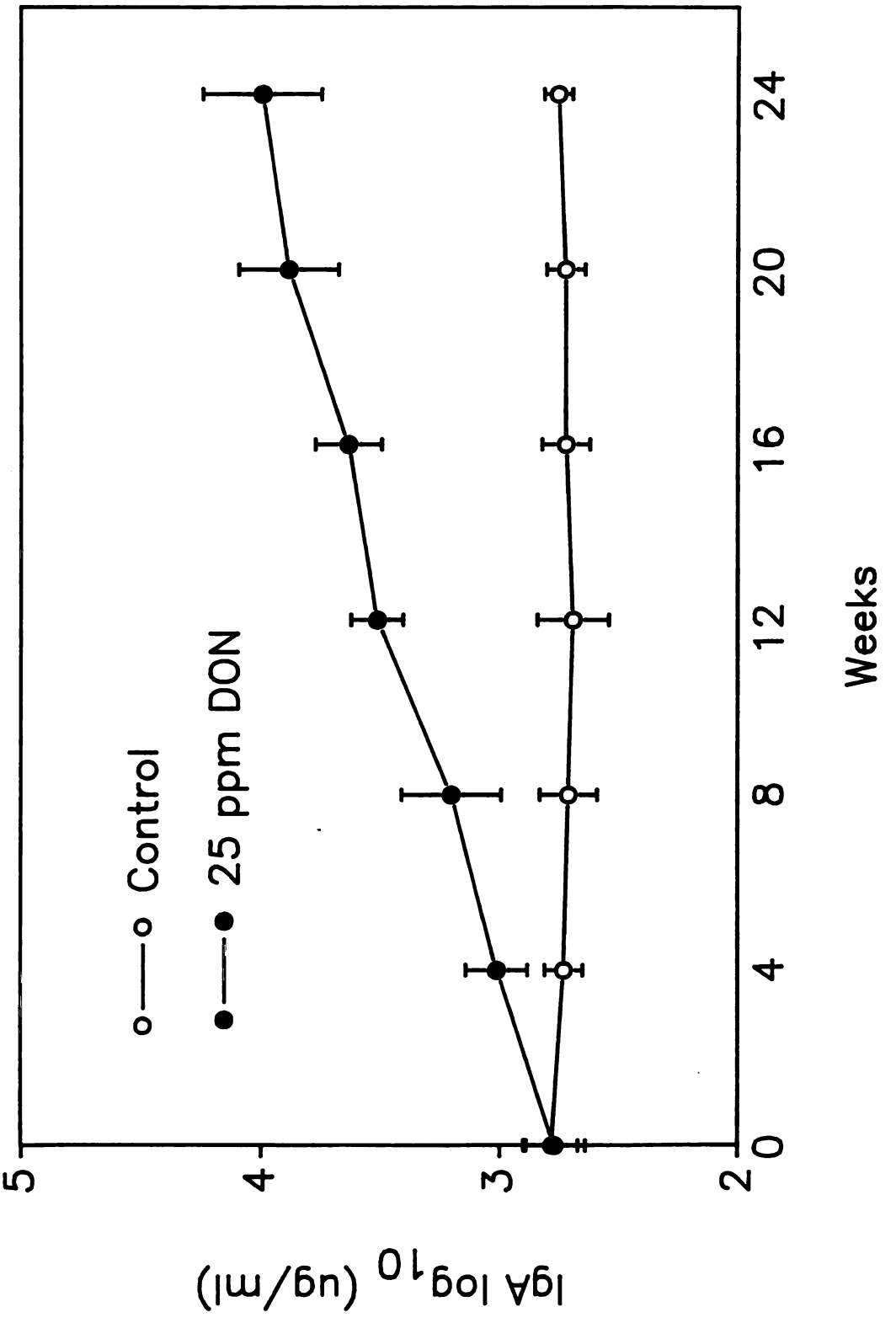
[a] Mice were fed 25 ppm DON in AIN-76A diet for 8 weeks. (Exp 5)

[b] Data shown are geometric mean ± standard deviation for groups of 11 ad lib control, 12 treated, and 11 restricted control mice. Difference between treatment and ad lib control determined by Dunnett's t test, and difference between treatment and restricted controls determined by the improved Bonferroni t test.

[c] Significantly different than restricted control ($p<0.01$).

[d] Significantly different than ad lib control ($p<0.01$).

Figure 3. Serum IgA following long term dietary DON exposure. Mice were fed 25 ppm DON in AIN-76A diet for 24 weeks. Data shown are geometric means (ug/ml) $\pm$ S.D. for groups of 13 mice. Difference between treatment and ad lib control group determined by Dunnett's t test. Treatment and control groups are significantly different from week 4 to week 24 ($p<0.01$).


IgA log10 (ug/ml)
5
4
3
2
0
4
8
12
16
20
24
Weeks
o—o Control
●—● 25 ppm DON

Salivary IgA concentrations in mice following dietary deoxynivalenol exposure. Since IgA serves a critical role as a mucosal immunoglobulin, it was of interest to know the salivary IgA concentrations following exposure to this dietary antigen. The immunoglobulin concentrations were measured in the saliva of Exp. 1 animals following 2 and 10 ppm dietary DON and are presented in Table 5. The 2 ppm DON group was significantly elevated ($p<0.05$) compared to its restricted control after 6 weeks of feeding. The 2 and 10 ppm DON groups were elevated relative to their restricted and ad lib controls at weeks 2, and 8 but were not significant due to the large amount of variation.

The effect of dietary DON on total and antigen-specific serum immunoglobulin response. Two studies were performed to determine if dietary DON could increase the antigen-specific IgA concentrations using cholera toxin and casein. Cholera toxin has been found to be an extremely potent immunogen for mucosal IgA responses when administered orally (Elson and Ealding, 1984). Casein was the protein source in the AIN-76A diet that the animals received for the duration of the study.

In the first antigen challenge study (Exp 3), mice were fed 10 ppm DON for 14 days and then orally challenged with 10 ug of cholera toxin. Animals were continued on the DON diet and boosted with 10 ug cholera toxin on days 28 and 41. At day 48, animals were sacrificed and serum and intestinal contents were collected. Table 6 presents the serum IgA concentrations in mice following 10 ppm DON and cholera toxin

Table 5. Salivary IgA concentration in mice following dietary exposure to 2 and 10 ppm DON.[a]

	IgA Log_{10}(ug/ml)[b]			
Treatment	wk 2	wk 4	wk 6	wk 8
2 ppm DON	1.09 ± 0.36 (182)	0.77 ± 0.35 (105)	1.05 ± 0.32[c] (195)	1.15 ± 0.43 (129)
2 Restricted	0.86 ± 0.37 (107)	0.64 ± 0.40 (78)	0.75 ± 0.30[c] (98)	0.91 ± 0.25 (74)
10 ppm DON	0.95 ± 0.36 (132)	0.74 ± 0.32 (98)	0.79 ± 0.32 (107)	1.09 ± 0.21 (112)
10 Restricted	0.77 ± 0.32 (87)	0.52 ± 0.15 (59)	0.74 ± 0.34 (95)	0.94 ± 0.31 (79)
ad lib control	0.83 ± 0.24	0.75 ± 0.27	0.76 ± 0.30	1.04 ± 0.36

[a] $B6C3F_1$ treatment mice were fed 2 or 10 ppm DON in AIN-76A diet. (Exp 1)

[b] Data shown are geometric mean ± standard deviation for groups of 22 control and 10 treated mice. Numbers in parentheses are (ratio of means of treatment to ad lib) X 100. Difference between treatment groups and ad lib control determined by Dunnett's t test. Difference between treatment groups and their restricted controls determined by the improved Bonferroni t test.

[c] Groups with identical letters are significantly different ($p<0.05$).

Table 6. Serum IgA in mice following exposure to 10 ppm DON and oral cholera toxin challenge.[a]

	IgA Log_{10} (ug/ml)[b]				
Treatment	Day 0	Day 14	Day 35	Day 41	Day 48
ad lib control	2.72 ± 0.07	2.71 ± 0.10	2.68 ± 0.05	2.68 ± 0.11	2.74 ± 0.08
10 ppm DON	2.69 ± 0.08	2.79 ± 0.10[c]	2.79 ± 0.15[c]	2.77 ± 0.12	2.77 ± 0.05
cholera control	2.68 ± 0.09	2.68 ± 0.05	2.75 ± 0.06	2.70 ± 0.09	2.85 ± 0.15
cholera-10 ppm DON	2.74 ± 0.08	2.82 ± 0.09[c,d]	2.81 ± 0.14[c]	2.70 ± 0.12	2.89 ± 0.15

[a] Mice were fed 10 ppm DON DON in AIN-76A diet for 48 days. Cholera toxin treatment animals were gavaged with 10 ug cholera toxin at day 14, and boosted on days 28, and 41. (Exp 3)

[b] Data shown are geometric mean values ± standard deviation for groups of 13 control, 12 10 ppm, 10 cholera control, and 15 cholera-10 ppm DON mice. Difference between treatment and control groups determined by Dunnett's t test. Difference between treatment groups and their matched controls determined by the Bonferronit test.

[c] Significantly different than ad lib control ($p<0.05$).

[d] Significantly different than matched cholera toxin control ($p<0.05$).

challenge. Unfortunately the DON effect on serum IgA was minimal in this experiment. The 10 ppm DON group was significantly increased ($p<0.05$) relative to the ad lib control at days 14 and 35. IgA concentrations in the cholera-10 ppm DON group were higher ($p<0.01$) than the ad lib control at days 14,35, and 48 and higher than the cholera control at day 14 ($p<0.05$). Table 7 presents the intestinal IgA concentrations following 10 ppm DON and cholera toxin challenge. Intestinal IgA concentrations were higher in the cholera-10 ppm DON group than the cholera control however this increase was not significant due to the large amount of variation.

Serum IgA concentrations were measured in mice that were fed 25 ppm DON and challenged with cholera toxin (Exp 6). Mice were fed DON for 5 weeks and then challenged with cholera toxin at wk 5, and boosted at weeks 7, and 21. Table 8 reveals that serum IgA was elevated ($p<0.01$) in the cholera-DON group relative to the cholera control and to the ad lib control at weeks 4, 8, and 12. Cholera challenge alone did not significantly alter the total serum IgA concentration, nor did it act synergistically with DON to increase the serum IgA concentration.

Cholera toxin and casein-specific serum IgA were measured in the 25 ppm DON-cholera toxin experiment (Exp 6). Table 9 and Fig. 4 present the ELISA titers for cholera toxin -specific IgA at weeks 8 and 21. Cholera toxin-specific antibody was measured at week 20 for the ad lib control and 25 ppm DON group, and at week 22 for the cholera toxin and

Table 7. Total intestinal IgA of mice following dietary DON and oral cholera toxin challenge.[a]

	IgA Log_{10}(ug/ml)[b]			
Treatment	control	10 ppm DON	cholera control	cholera-10 ppm DON
Day 48	1.29 ± 0.48	1.22 ± 0.39	1.13 ± 0.53	1.39 ± 0.44

[a] Mice were fed 10 ppm DON in AIN-76 diet for 48 days. Cholera toxin treatment animals were gavaged with 10 ug cholera toxin on day 14, and boosted on days 28, and 41. (Exp 3)

[b] Data shown are geometric mean ± standard deviation for groups of 13 control, 12 10 ppm, 10 cholera control, and 15 cholera-10 ppm DON mice. Difference between groups determined by Student's t test.

Table 8. Serum IgA in mice following exposure to 25 ppm dietary DON and oral cholera toxin challenge.[a]

	IgA Log_{10}(ug/ml)[b]						
Treatment	wk 0	wk 4	wk 8	wk 12	wk 16	wk 20	wk 24
ad lib control	2.78 ± 0.11	2.73 ± 0.08	2.71 ± 0.12	2.69 ± 0.15	2.72 ± 0.10	2.72 ± 0.08	2.75 ± 0.06
25 ppm DON	2.77 ± 0.13	3.01 ± 0.13[c]	3.20 ± 0.21[c]	3.51 ± 0.11[c]	3.63 ± 0.14[c]	3.88 ± 0.21[c]	3.99 ± 0.25[c]
Cholera Toxin	2.75 ± 0.13	2.75 ± 0.08	2.64 ± 0.07	2.67 ± 0.27	—	—	—
Cholera 25 ppm DON	2.75 ± 0.11	2.98 ± 0.11[c,d]	3.24 ± 0.23[c,d]	3.61 ± 0.24[c,d]	—	—	—

[a] Mice were fed 25 ppm DON in AIN-76A diet for 24 weeks. Cholera toxin treatment animals were gavaged with 10 ug cholera toxin at week 5, and boosted at weeks 7, and 21. (Exp 6)

[b] Data shown are geometric mean ± standard deviation for groups of 13 mice. Cholera 25 ppm DON group consisted of 14 mice. Difference between treatment and control groups determined by Dunnett's t test. Difference between treatment groups and their matched controls determined by the Bonferroni t test.

[c] Significantly different than ad lib control ($p<0.01$).

[d] Significantly different than matched cholera toxin control ($p<0.01$).

Table 9. Cholera toxin and Casein-specific serum IgA following dietary DON and oral cholera toxin challenge.[a]

	cholera specific IgA Log_{10}(ng/ml)[b]				casein specific IgA Log_{10}(ng/ml)[b]			
Treatment	wk 8	wk 16	wk 20	wk 22	wk 8	wk 16	wk 20	wk 22
ad lib control	3.30 ± 0.00	3.14 ± 0.13	3.02 ± 0.09	——	3.43 ± 0.20	3.56 ± 0.15	3.47 ± 0.31	——
25 ppm DON	3.42 ± 0.14	3.90 ± 0.36[c]	3.72 ± 0.27[c]	——	3.58 ± 0.27	4.56 ± 0.39[c]	4.57 ± 0.46[c]	——
cholera control	3.67 ± 0.33[c]	——	——	4.47 ± 0.84	3.34 ± 0.11	——	——	3.44 ± 0.40
cholera-25 ppm DON	3.63 ± 0.45[c,d]	——	——	4.40 ± 0.63	3.60 ± 0.24[d]	——	——	4.95 ± 0.53[d]

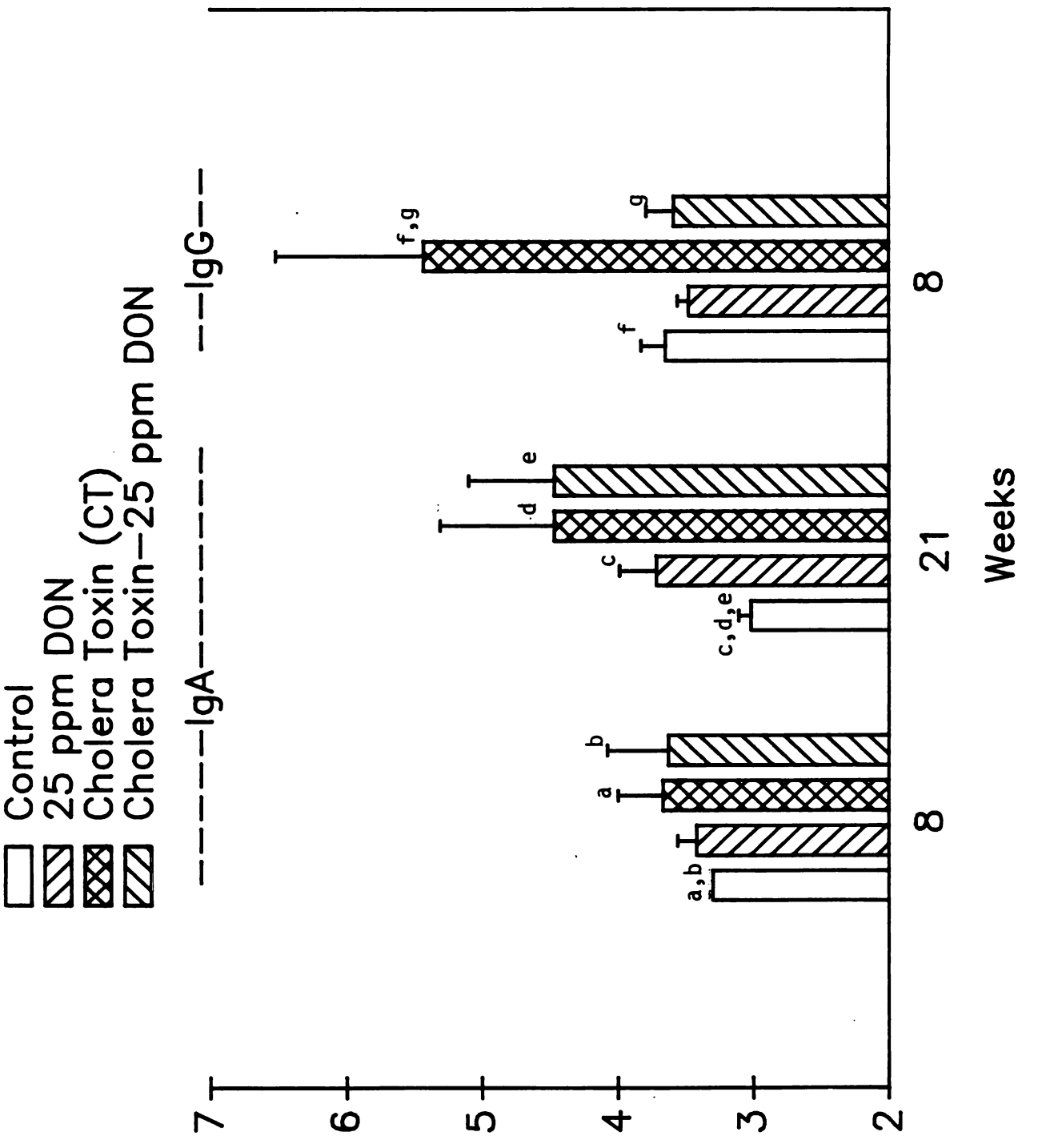
[a] Mice were fed 25 ppm DON in AIN-76A diet for 22 weeks. Cholera toxin treatment animals were gavaged with 10 ug cholera toxin at week 5, and boosted at weeks 7, and 21. (Exp 6)

[b] Data shown are geometric mean ± standard deviation for groups of 13 mice. Cholera 25 ppm DON group had 14 mice. Values determined by ELISA. Absorbances for IgA specific antibody were equated to absorbances from mouse reference serum and equivalent values were assigned. Difference between treatment and ad lib control determined by Dunnett's t test. Difference between DON treatment and matched control determined by Bonferroni t test.

[c] Significantly different than ad lib control ($p<0.01$).

[d] Significantly different than matched cholera toxin control ($p<0.01$).

Figure 4. Cholera toxin-specific equivalent IgA and IgG concentrations following dietary DON and cholera toxin challenge. Mice were fed 25 ppm DON for 24 weeks and challenged with 10 ug cholera toxin/mouse at wk 5, and boosted at wks 7 and 21. Values determined by ELISA. Absorbances for immunoglobulin specific antibody were equated to absorbances from dilutions of mouse reference serum run concurrently and equivalent concentration values were assigned. Data shown are geometric mean (ng/ml) $\pm$ S.D. for groups of 13 mice. The cholera-25 ppm DON group had 14 mice. Difference between treatment and ad lib control determined by Dunnett's t test. Difference between DON treatment and matched control determined by Bonferroni t test. Bars with identical letters are significantly different ($p<0.01$).


Control
25 ppm DON
Cholera Toxin (CT)
Cholera Toxin—25 ppm DON
IgA
IgG
CT specific Ig Log10 (ng/ml)
7
6
5
4
3
2
a,b
a
b
c
c,d,e
d
e
f
f,g
g
8
21
8
Weeks

cholera toxin-25 ppm DON group. The means of these groups were statistically analyzed by Dunnett's t test and grouped together as week 21 in figure 5. Cholera toxin specific IgA levels in the immunized DON treated animals were elevated ($p<0.01$) 5 fold at week 21 when compared to the ad lib control. Interestingly, cholera toxin specific IgA at this time was not elevated in the DON group that was challenged with cholera toxin when compared to the cholera control. Both cholera toxin groups were elevated ($p<0.01$) relative to the ad lib control at weeks 8 and 21. The cholera toxin-specific IgA titer in the unimmunized control fed 25 ppm DON was significantly elevated relative to the ad lib control.

Casein-specific IgA data were pooled at weeks 20 and 22 in the same manner as the cholera toxin specific IgA (Fig.5). Following 21 weeks of dietary DON, casein-specific IgA was significantly elevated ($p<0.01$) in the 25 ppm DON treatment groups relative to their ad lib controls for both cholera toxin immunized and unimmunized groups (Table 9 and Fig. 5). A 32-fold difference in DON-treated animals was observed in immunized animals relative to the ad lib control. This same profile was seen at week 8 in the immunized groups. The antigen-specific equivalent IgA level and total IgA concentration in the intestine are presented in Table 11. The casein-specific equivalent IgA level and the total IgA concentration were increased ($p<0.05$) in the cholera toxin-25 ppm DON group, however the latter was not significant. Cholera toxin-specific equivalent IgA level was decreased in mice fed 25 ppm DON and challenged with cholera toxin

Figure 5. Casein-specific equivalent IgA and IgG concentrations following dietary DON and cholera toxin challenge. Mice were fed 25 ppm DON for 24 weeks and challenged with 10 ug cholera toxin/mouse at wk 5, and boosted at wks 7 and 21. Values determined by ELISA. Absorbances for immunoglobulin specific antibody were equated to absorbances from dilutions of mouse reference serum run concurrently and equivalent concentration values were assigned. Data shown are geometric mean (ng/ml) $\pm$ S.D. for groups of 13 mice. The cholera-25 ppm DON group had 14 mice. Difference between treatment and ad lib control determined by Dunnetts't test. Difference between DON treatment and matched control determined by Bonferroni t test. Bars with identical letters are significantly different ($p<0.01$).

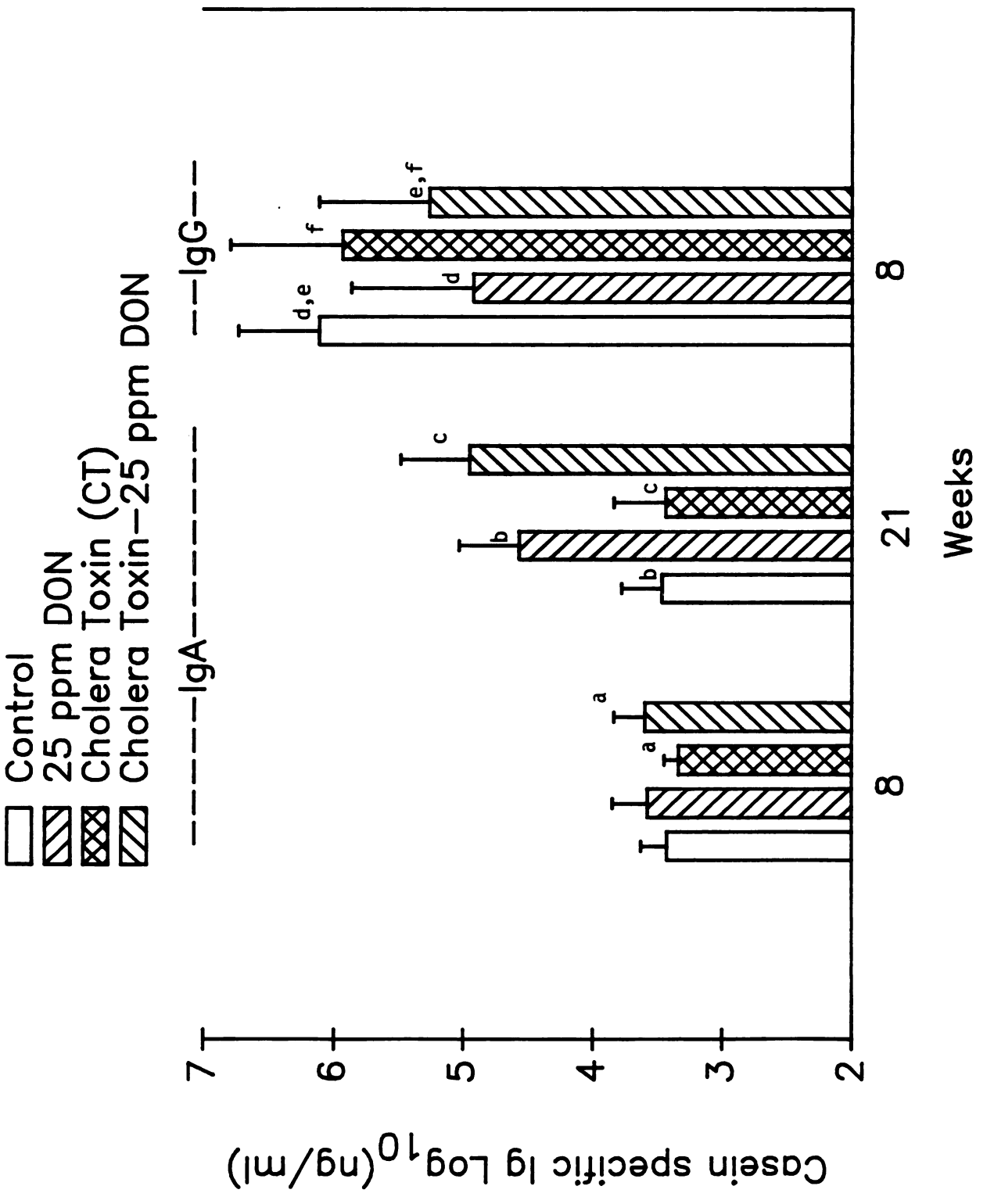

Control
25 ppm DON
Cholera Toxin (CT)
Cholera Toxin—25 ppm DON
—IgA—
—IgG—
Casein specific Ig Log10(ng/ml)
7
6
5
4
3
2
a
a
b
b
c
c
d
d,e
f
e,f
8
21
8
Weeks

Table 10. Cholera and Casein-specific serum IgG following 8 weeks of dietary DON and oral cholera toxin challenge.[a]

	Antigen specific IgG Log_{10}(ng/ml)[b]	
Treatment	Cholera toxin	Casein
ad lib control	3.65 ± 0.18	6.11 ± 0.62
25 ppm DON	3.48 ± 0.08	4.92 ± 0.94[c]
cholera control	5.43 ± 1.09[c]	5.93 ± 0.86
cholera-25 ppm DON	3.59 ± 0.20[d]	5.26 ± 0.85[c,d]

[a] Mice were fed 25 ppm DON in AIN-76A diet for 8 weeks. Cholera toxin treatment animals were gavaged with 10 ug cholera toxin at week 5, and boosted at week 7. (Exp 6)

[b] Data shown are geometric mean ± standard deviation for groups of 13 mice. The cholera-25 ppm DON group had 14 mice. Values determined by ELISA. Absorbances for IgG specific antibody were equated to absorbances from mouse reference serum and equivalent values were assigned. Difference between treatment and ad lib control determined by Dunnett's t test. Difference between DON treatment and matched control determined by Bonferroni t test.

[c] Significantly different than ad lib control ($p<0.01$).

[d] Significantly different than matched cholera toxin control ($p<0.01$).

Table 11. Total and antigen-specific intestinal IgA following 22 weeks of dietary DON and oral cholera toxin challenge.[a]

	Antigen specific IgA Log_{10}(ng/ml)[b]		
Treatment	Cholera toxin	Casein	Total IgA
cholera control	3.68 ± 1.18	1.70 ± 0.00[c]	2.51 ± 0.22
cholera-25 ppm DON	2.45 ± 0.71	1.99 ± 0.33[c]	2.66 ± 0.24

[a] Mice were fed 25 ppm DON in AIN-76A diet for 8 weeks. Cholera toxin treatment animals were gavaged with 10 ug cholera toxin at week 5, and boosted at week 7 and 21. (Exp 6)

[b] Data shown for antigen specific IgA are geometric mean ± standard deviation for groups of 13 mice. The cholera-25 ppm DON group had 14 mice. Values determined by ELISA. Absorbances for IgG specific antibody were equated to absorbances from mouse reference serum and equivalent values were assigned. Values for total IgA were determined by ELISA with concentrations determined from mouse reference serum. Difference between treatments determined by Students t test

[c] Values with identical letters are significantly different ($p<0.05$)

relative to the cholera toxin control but this was not significant due to the large amount of variation.

Cholera toxin and casein-specific IgA responses correspond to the total IgA profile generated upon chronic dietary DON exposure. Serum was analyzed for cholera toxin and casein specific IgG at week 8 to see if this immunoglobulin also mimics the decrease in total levels found following DON exposure (Table 10, Fig.4,5). Cholera toxin challenge elevates cholera toxin specific IgG ($p<0.01$) as has been described previously (Elson and Ealding, 1984). This effect was suppressed when animals were fed 25 ppm DON and challenged with cholera toxin ($p<0.01$). Casein-specific IgG concentrations were also significantly decreased ($p<0.05$) in animals that received dietary DON regardless of cholera toxin challenge. Casein specific IgG levels were the same in the cholera toxin control and in the ad lib control. These results suggest that the dietary DON is responsible for the decrease in casein specific IgG and not due to the co-administration of DON and cholera toxin.

To validate the specificity of the antigenic-specific ELISA's, fractions of serum were pooled from mice at week 22 that were treated with 25 ppm DON and challenged with cholera toxin. Absorbance decreased 67% with 100 ug/ml free cholera toxin in the assay. Fractions of serum were likewise pooled after 20 weeks of 25 ppm DON and tested for casein specific IgA. Absorbance was decreased 59% with 500 ug/ml free casein.

<u>In vitro</u> immunoglobulin production by isolated

splenocytes following dietary DON exposure. In vitro immunoglobulin production by isolated splenocytes was evaluated to assess the contribution of the systemic compartment to elevated serum IgA following DON exposure The objective of these in vitro studies were to demonstrate that the spleen was involved with the elevated serum IgA presented in earlier in vivo studies.

Mitogens are commonly used to induce lymphocyte activation. LPS and Con A are B and T cell lymphocyte mitogens respectively and were employed to stimulate the lymphocytes and induce immunoglobulin secretion. Table 12 indicates that IgA production by isolated splenocytes was markedly affected by DON exposure. Of 5 trials performed to demonstrate the in vitro immunoglobulin production, trial 2 was selected as most representative of the in vitro studies and is presented in figure 6. Here LPS stimulated lymphocytes from DON-treated animals exhibited a 400% increase in IgA production relative to the restricted and ad lib control lymphocytes. This same profile was seen in the Con A and Con A-LPS stimulated lymphocytes. Interestingly, there was an even greater total increase in IgA production from DON treated animals compared to the restricted and ad lib control in spontaneous (non-mitogen stimulated) lymphocytes. This is shown by the stimulation index in table 12 which describes the IgA produced by the combined effect of mitogen and/or treatment relative to the spontaneous ad lib control.

Table 13 shows in vitro IgG production by isolated

Table 12. Effect of dietary DON on in vitro IgA production by isolated splenic lymphocytes[a]

	IgA concentration Log_{10}(ng/ml)[b]							
	LPS		Con A		Con A-LPS		Spontaneous	
Trial	DON	ad lib	DON	ad lib	DON	ad lib	DON	ad lib
1	4.26 ± 0.12[d]	3.31 ± 0.08	3.76 ± 0.11[d]	2.38 ± 0.16	3.93 ± 0.18[d]	2.46 ± 0.17	4.30 ± 0.00[d]	2.89 ± 0.34
(S.I.)[c]	(23.44)	(2.63)	(7.41)	(0.31)	(10.96)	(0.37)	(25.70)	
2	4.60 ± 0.00[d]	3.71 ± 0.30	3.84 ± 0.06[d]	2.91 ± 0.14	3.82 ± 0.15[d]	3.07 ± 0.23	4.48 ± 0.16[d]	3.33 ± 0.28
	(18.62)	(2.40)	(3.24)	(0.38)	(3.09)	(0.55)	(14.13)	
3	4.30 ± 0.00[d]	3.47 ± 0.18	3.34 ± 0.17	3.59 ± 0.40	3.37 ± 0.18	3.59 ± 0.10	3.43 ± 0.10[d]	2.51 ± 0.19
	(61.66)	(9.12)	(6.76)	(12.02)	(7.24)	(12.02)	(8.32)	
4	4.06 ± 0.12	3.76 ± 0.12	3.77 ± 0.08	3.23 ± 0.21	3.81 ± 0.02[d]	3.33 ± 0.05	3.91 ± 0.08[d]	3.18 ± 0.17
	(7.59)	(3.80)	(3.89)	(1.12)	(4.27)	(1.41)	(5.37)	
5	4.33 ± 0.00[d]	3.72 ± 0.11	3.31 ± 0.02[d]	2.82 ± 0.14	3.79 ± 0.16[d]	3.10 ± 0.03	3.78 ± 0.13[d]	2.77 ± 0.27
	(36.31)	(8.91)	(3.47)	(1.12)	(10.47)	(2.14)	(10.23)	

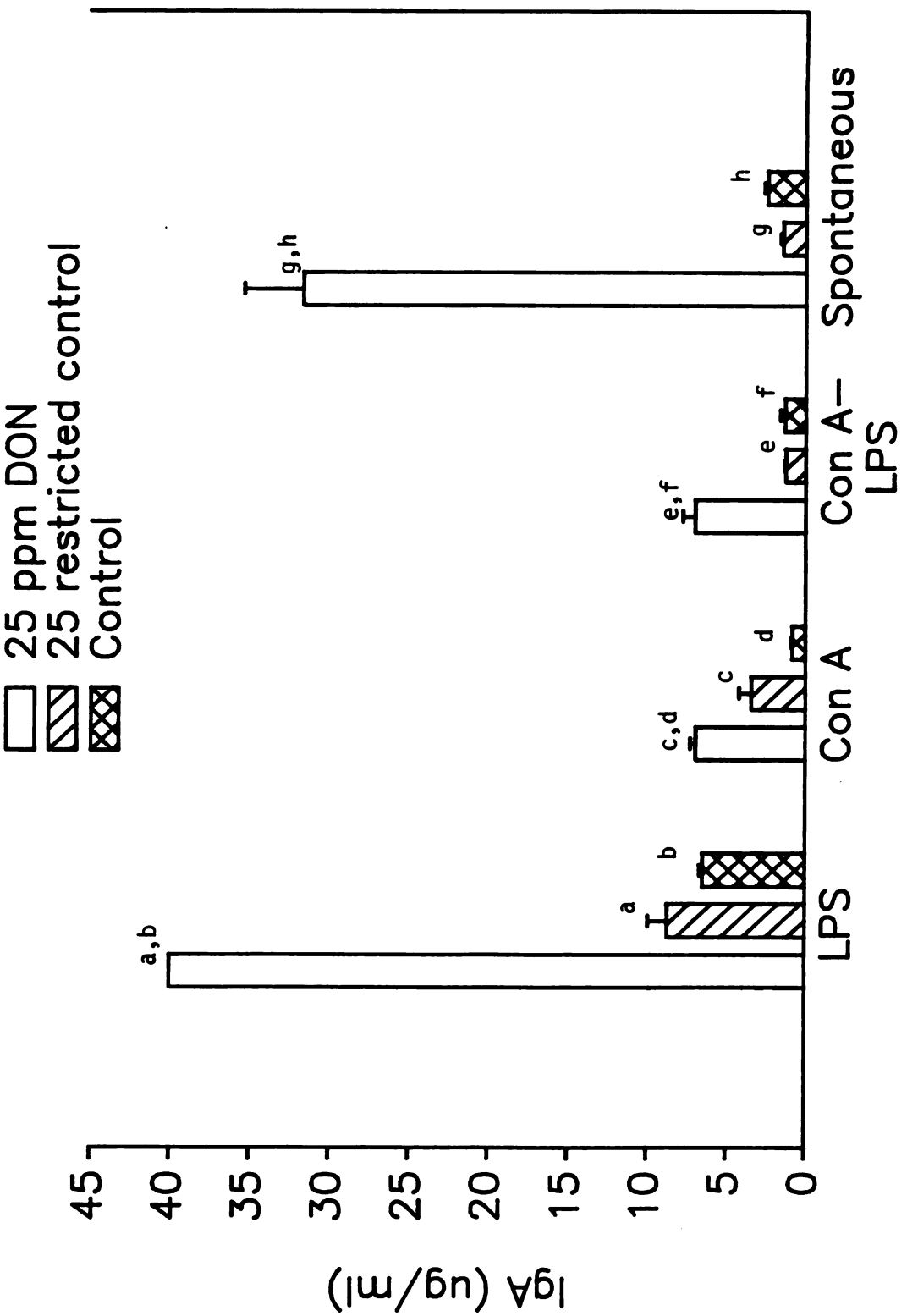
[a] $B6C3F_1$ mice (2/trial) were fed 25 ppm DON or clean diet for 21 weeks (trial 1,2) or 22 weeks (trial 3,4,5). Spleen cell suspensions (5×10^5 cells/ml/well) were prepared in 20% FCS-RPMI-1640 and exposed to LPS (20 ug/ml), Con A (10 ug/ml), Con A-LPS (10 and 20 ug/ml) or water for seven days at 37°C-5% CO_2. Supernatants were analyzed for IgA by ELISA.

[b] Data shown are geometric mean (Log_{10}) ± S.D.. In trials 1 and 2, ELISA performed in duplicate for 4 wells from each trial. Value represents mean of 8 determinations. In trials 3, 4, and 5, supernatants from 3 wells from each trial were assayed in duplicate. Value represents mean of 6 determinations.

[c] Stimulation Index (S.I.) = treatment value/spontaneous ad lib value

[d] Significantly different than intra-mitogen ad lib control ($p<0.05$)

Figure 6. Effect of dietary DON on *in vitro* IgA production by isolated splenocytes. $B6C3F_1$ mice (2/trial) were fed 25 ppm DON for 21 weeks. Restricted groups were fed AIN-76A diet at level equivalent to mean intake of 25 ppm DON group. Spleen cell suspensions (5 x 10^5 cells/well) were prepared in 20% RPMI-1640 and exposed to LPS (20 ug/ml), Con A (10 ug/ml), Con A-LPS (10 and 20 ug/ml) or water for seven days at 37^oC-5% CO_2. Supernatants were analyzed for IgA by ELISA. Data shown are mean (ug/ml) $\pm$ S.E.. Groups with identical letters are significantly different ($p<0.01$). Data for figure from trial 2 of table 12.


25 ppm DON
25 restricted control
Control
IgA (ug/ml)
45
40
35
30
25
20
15
10
5
0
a,b
a
b
c,d
c
d
e,f
e
f
g,h
g
h
LPS
Con A
Con A— LPS
Spontaneous

lymphocytes. Three experiments were analyzed for IgG with trial 2 being most representative and presented in figure 7. As expected, LPS- activated lymphocytes produced the largest amount of immunoglobulin. With all the mitogens and the spontaneous control, no significant difference was seen between lymphocytes from DON treated animals and the ad lib control. Contrary to the IgA profile, spontaneous IgG production was very small.

In vitro IgM production by isolated lymphocytes is presented in table 14. Three experiments were measured for IgM with trial 2 shown in figure 8. IgM production was significantly decreased in single mitogen stimulated cultures from DON treated animals relative to the ad lib control ($p<0.05$). Again, very little IgM was produced in the spontaneous cultures.

Table 13. Effect of dietary DON on in vitro IgG production by isolated splenic lymphocytes[a]

	IgG concentration Log_{10}(ng/ml)[b]							
	LPS		Con A		Con A-LPS		Spontaneous	
Trial	DON	ad lib	DON	ad lib	DON	ad lib	DON	ad lib
1	3.77 ± 0.03	3.63 ± 0.09	2.69 ± 0.09	2.22 ± 0.23	—	—	2.84 ± 0.01	3.07 ± 0.32
(S.I.)[c]	(5.01)	(3.63)	(0.42)	(0.14)			(0.59)	
2	3.63 ± 0.28	3.61 ± 0.02	2.48 ± 0.09	2.47 ± 0.10	2.96 ± 0.05	2.93 ± 0.12	2.44 ± 0.05	2.44 ± 0.10
	(15.49)	(14.79)	(1.10)	(1.07)	(3.31)	(3.09)	(1.00)	
3	3.95 ± 0.12	2.76 ± 0.05	2.66 ± 0.18	3.01 ± 0.17	3.01 ± 0.24	3.03 ± 0.04	2.62 ± 0.34	2.75 ± 0.12
	(15.85)	(0.23)	(0.81)	(1.82)	(1.82)	(1.91)	(0.74)	

[a] $B6C3F_1$ mice (2/trial) were fed 25 ppm DON or clean diet for 21 weeks (trial 1,2) or 22 weeks (trial 3). Spleen cell suspensions (5×10^5 cells/ml/well) were prepared in 20% FCS-RPMI-1640 and exposed to LPS (20 ug/ml), Con A (10 ug/ml), Con A-LPS (10 and 20 ug/ml) or water for seven days at 37°C-5% CO_2. Supernatants were analyzed for IgG by ELISA.

[b] Data shown are geometric mean (Log_{10}) ± S.D.. In trial 1, assay performed in duplicate for 4 wells. Value represents mean of 8 determinations. In trials 2 and 3, assay performed in duplicate for 3 wells. Duplicates were averaged with the value representing the mean of 3 determinations.

[c] Stimulation Index (S.I.) = treatment value/spontaneous ad lib value

[d] Significantly different than intra-mitogen ad lib control ($p<0.05$)

Figure 7. Effect of dietary DON on *in vitro* IgG production by isolated splenocytes. $B6C3F_1$ mice (2/trial) were fed 25 ppm DON for 21 weeks. Spleen cell suspensions (5×10^5 cells/well) were prepared in 20% RPMI-1640 and exposed to LPS (20 ug/ml), Con A (10 ug/ml), Con A-LPS (10 and 20 ug/ml) or water for seven days at $37^{\circ}C$-5% CO_2. Supernatants were analyzed for IgG by ELISA. Data shown are mean (ug/ml) $\pm$ S.E.. Groups with identical letters are significantly different ($p<0.01$). Data for figure from trial 2 of table 13.

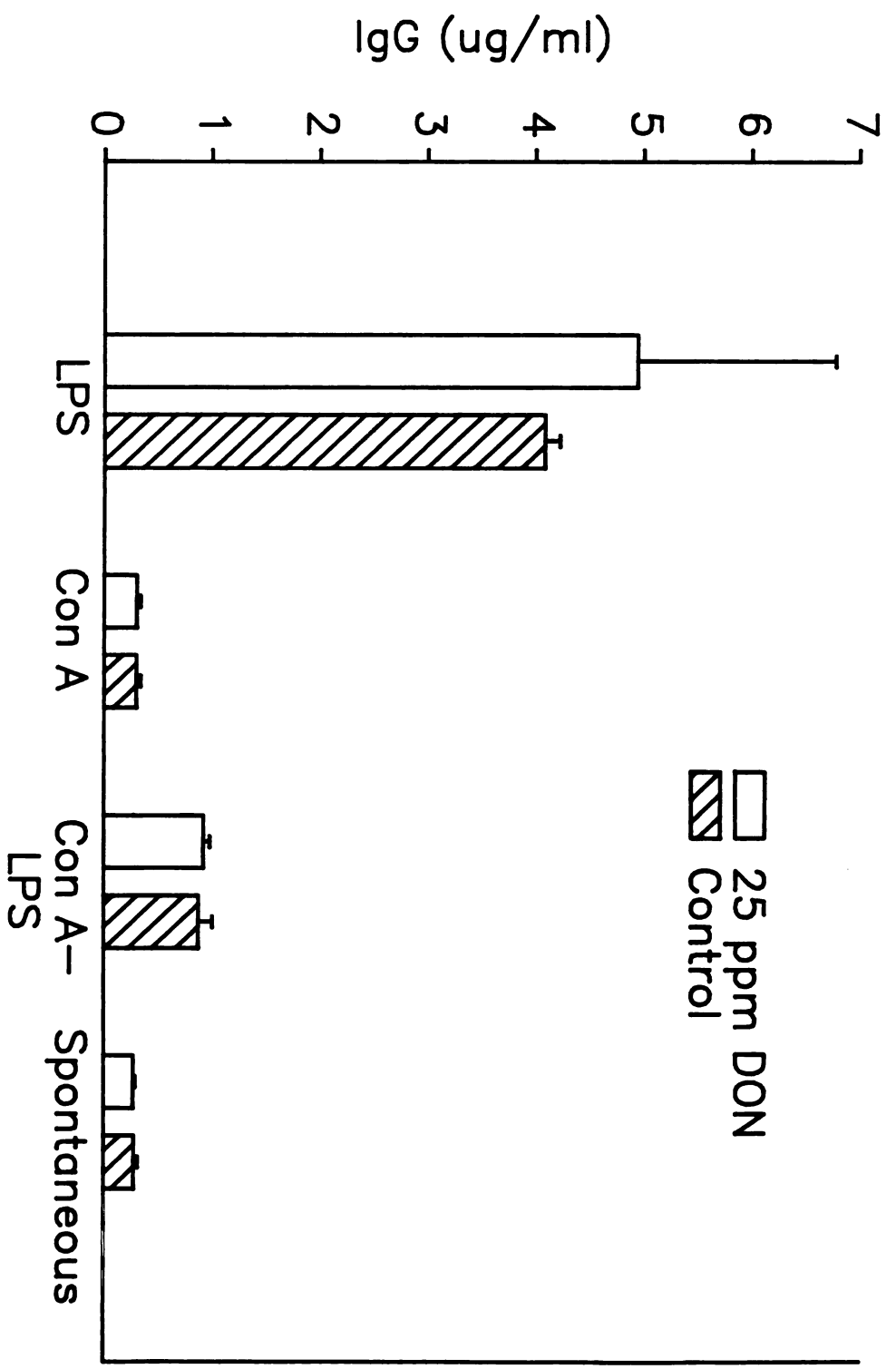

IgG (ug/ml)
0
1
2
3
4
5
6
7
LPS
Con A
Con A—
LPS
Spontaneous
25 ppm DON
Control

Table 14. Effect of dietary DON on in vitro IgM production by isolated splenic lymphocytes[a]

	IgM concentration Log_{10}(ng/ml)[b]							
	LPS		Con A		Con A-LPS		Spontaneous	
Trial	DON	ad lib	DON	ad lib	DON	ad lib	DON	ad lib
1	4.13 ± 0.00	4.30 ± 0.09	2.76 ± 0.07	2.58 ± 0.31	—	—	3.47 ± 0.29	3.62 ± 0.39
(S.I.)[c]	(3.24)	(4.79	(0.14)	(0.09)			(0.71)	
2	3.60 ± 0.13[d]	4.18 ± 0.09	2.95 ± 0.03[d]	3.50 ± 0.20	3.74 ± 0.03[d]	4.03 ± 0.05	2.47 ± 0.08	2.67 ± 0.11
	(8.51)	(32.36)	(1.91)	(6.76)	(11.75)	(22.91)	(0.63)	
3	4.04 ± 0.02	4.12 ± 0.08	2.85 ± 0.16	3.20 ± 0.06	3.88 ± 0.09	4.11 ± 0.07	2.50 ± 0.11	2.79 ± 0.10
	(17.78)	(21.38)	(1.15)	(2.57)	(12.30)	(20.89)	(0.51)	

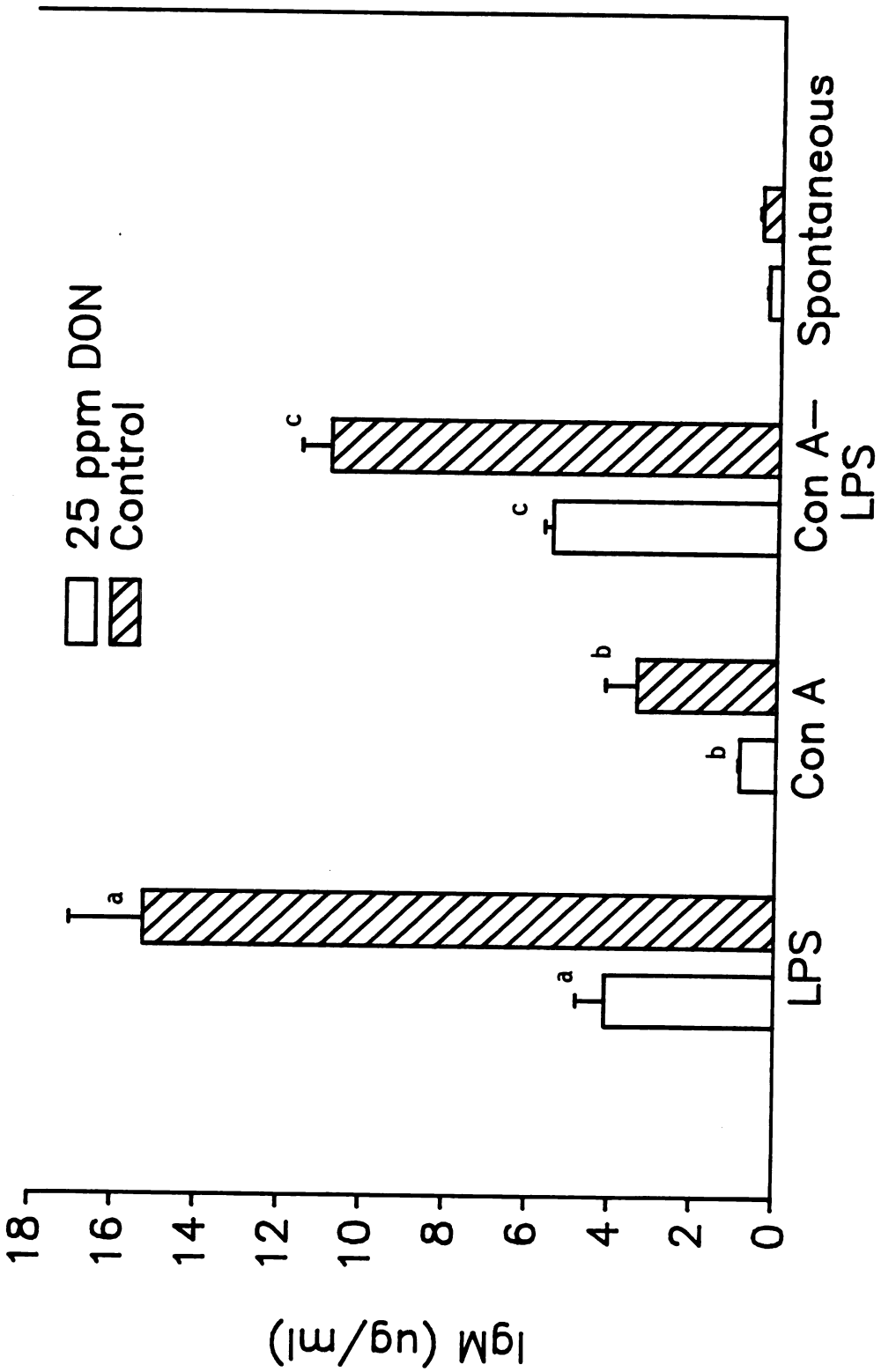
[a] $B6C3F_1$ mice (2/trial) were fed 25 ppm DON or clean diet for 21 weeks (trial 1,2) or 22 weeks (trial 3,4,5). Spleen cell suspensions (5×10^5 cells/ml/well) were prepared in 20% FCS-RPMI-1640 and exposed to LPS (20 ug/ml), Con A (10 ug/ml), Con A-LPS (10 and 20 ug/ml) or water for seven days at 37°C-5% CO_2. Supernatants were analyzed for IgM by ELISA.

[b] Data shown are geometric mean (Log_{10}) ± S.D.. In trial 1, assay performed in duplicate for 4 wells. Value represents mean of 8 determinations. In trials 2 and 3, assay performed in duplicate for 3 wells. Duplicates were averaged with the value representing the mean of 3 determinations.

[c] Stimulation Index (S.I.) = treatment value/spontaneous ad lib value

[d] Significantly different than intra-mitogen ad lib control ($p<0.05$)

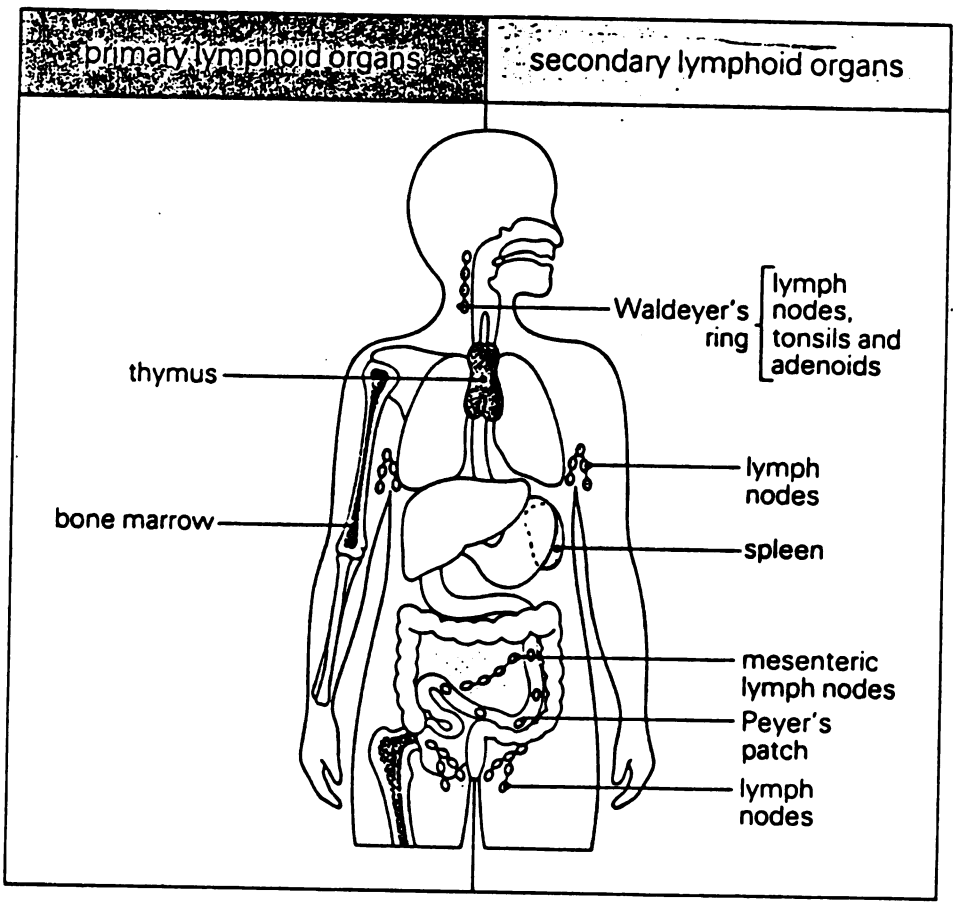
Figure 8. Effect of dietary DON on *in vitro* IgM production by isolated splenocytes. $B6C3F_1$ mice (2/trial) were fed 25 ppm DON for 21 weeks. Spleen cell suspensions (5×10^5 cells/well) were prepared in 20% RPMI-1640 and exposed to LPS (20 ug/ml), Con A (10 ug/ml), Con A-LPS (10 and 20 ug/ml) or water for seven days at $37^{o}C$-5% CO_2. Supernatants were analyzed for IgM by ELISA. Data shown are mean (ug/ml) $\pm$ S.E.. Groups with identical letters are significantly different ($p<0.01$). Data for figure from trial 2 of table 14.


IgM (ug/ml)
18
16
14
12
10
8
6
4
2
0
25 ppm DON
Control
LPS
Con A
Con A— LPS
Spontaneous
a
b
c

DISCUSSION

Immunoglobulin is a major weapon of the immune response to infection. Immunoglobulin responses involve binding and neutralization of infectious agents as well as interaction with other cell types that initiate various immune reactions. Since the greatest exposure the host has to foreign antigens occurs in the gut, specialized lymphoid tissues and cell networks are located here to respond to antigens thus preventing harm to the host. Figure 9 demonstrates the major organs involved in an immune response, and the route of circulation of lymphocytes between the tissues (Male, 1986). Peyer's patches are specialized pools of lymphocytes that line the small intestine. Here specialized M cells within the Peyer's patches function to sample antigen absorbed through the intestine. This antigen, still fully immunogenic, is exposed to the underlying B and T cells of the Peyer's patch. A significant percentage of B cells in the Peyer's patch are committed to IgA production. The antigen-stimulated B cells migrate from the Peyer's patch through the mesenteric lymph nodes to the thoracic duct and ultimately into circulation. During this time, the B cells mature to IgA producing plasma cells or memory cells which eventually will "home" to distant mucosal tissues. Here they produce IgA specific for the antigen that caused stimulation in the Peyer's patch.

Figure 9.Major organs involved in an immune response and the route of circulation of lymphocytes between the tissues (Male, 1986).


primary lymphoid organs
secondary lymphoid organs
Waldeyer's ring
lymph nodes, tonsils and adenoids
thymus
lymph nodes
bone marrow
spleen
mesenteric lymph nodes
Peyer's patch
lymph nodes

Immunoglobulin A (IgA) is the major immunoglobulin at mucosal surfaces where it functions as a first line of defense. Here it binds and neutralizes antigen before it can be absorbed and cause harm. The function of IgA in serum is however unclear relative to its critical role in secretions. Immunoglobulin G (IgG) and immunoglobulin M (IgM) are the major serum immunoglobulins whereas IgA accounts for only 15-20% of total immunoglobulin (Roit et al., 1985). In humans the majority of serum IgA does not enter external secretions in significant amounts. It has been hypothesized that IgA in serum exists only to bind absorbed antigen that wasn't cleared at the mucosal surfaces.

The work presented here provides strong evidence that DON exerts a specific effect on IgA. The decrease in serum IgG and IgM observed here and previously (Forsell et al., 1986) upon dietary DON exposure is consistent with a potent protein synthesis inhibitor. The inclusion of restricted controls demonstrated that the decrease in serum IgG can be partially explained by decreased feed intake caused by DON.

Work performed by Forsell et al., (1986) demonstrated that 8-week dietary DON causes a dose-dependent increase in serum IgA. The threshold DON level for this increase was 2 ppm, whereas maximum IgA was found at 10 ppm. IgA at 25 ppm was significantly greater than the control but less than at 10 ppm. The purpose of this thesis was to develop a model that could be used to study mechanisms by which dietary DON alters serum immunoglobulin levels. The first experiment did not show elevated IgA following 8 weeks of 2 ppm dietary DON

although 10 ppm DON did significantly elevate serum IgA in experiment 2 from weeks 4 through 8. This verified the work of Forsell et al. (1986) and demonstrated that the increase in IgA can be seen as early as 4 weeks of dietary DON. Subsequent experiments demonstrated an even greater increase (17 fold at 24 weeks) in serum IgA at 25 ppm DON which expands upon the previous study. There is a possible explanation for the difference between DON levels where maximum serum IgA were found in the earlier work (10 ppm) by Forsell and that (25 ppm) reported here. The treatment animals in the Forsel study were less robust and appeared "more sickly" than animals in these studies (M.F. Witt, personal communication). A prior or ongoing mucosal infection in the animals of the Forsell study could have conceivably caused a much larger IgA response thus making the animals more sensitive to DON effects. IgA levels in the control mice from the Forsell study were 2-fold higher than an average of the control mice reported in this thesis. Although serum IgA from the previous study was quantitated by the ELISA method, these levels were again verified in frozen serum samples by the RID procedure performed for this thesis. In a preliminary experiment not reported in this thesis, animals were challenged with sheep red blood cells and keyhole limpet hemocyanin concurrently with dietary DON in an attempt to increase the control levels of serum IgA. This protocol was unsuccessful in elevating the control levels of serum IgA to those reported in the Forsell study.

To better assess the affect of DON on the antigen-

specific IgA response, cholera toxin and dietary casein were used as model antigens. Cholera toxin has been found to be an extremely potent immunogen for mucosal IgA responses when administered orally. When fed 10 ug cholera toxin, mice exhibit cholera toxin-specific IgA in intestinal secretions and substantial increases in cholera toxin-specific plasma IgG and IgA levels (Elson and Ealding, 1984). When the mice in this thesis were fed DON for 21 weeks and simultaneously challenged with cholera toxin, there was similar elevation in cholera toxin-specific serum IgA, but no difference was detectable between the cholera toxin or cholera toxin-25 ppm DON groups. It is thus apparent that the ability of cholera toxin to elicit a serum IgA response was not effected by DON. However cholera toxin-specific IgG concentrations were dramatically decreased in animals receiving dietary DON. This is likely a reflection of DON induced reduction of total IgG.

Total IgA concentrations and antigen-specific equivalent IgA levels in the intestine correspond to those reported in the serum. There was a trend toward higher casein-specific as well as total IgA in the cholera toxin-DON treated mice than in mice treated with cholera toxin only. However this was not significant due to the large amount of variation. Nevertheless the results do suggest that DON treatment does not decrease gut IgA.

Casein-specific serum IgA concentrations were sharply increased in animals receiving DON. The opposite profile was observed with casein specific IgG. Casein is normally

digested in the gastrointestinal tract and absorbed as small peptides. These small peptides are then further metabolized to carbon and nitrogen sources for metabolic activities in the cell. Forsell et al., (1987) showed that acute doses of DON resulted in extensive necrosis of the gastrointestinal tract. It is possible that increased absorption of casein due to DON induced increased intestinal permeation is responsible for an increased IgA response. This is consistent with the total IgA profile generated upon dietary DON exposure. The decrease in casein-specific serum IgG also parallels the total IgG profile, and like cholera toxin may be explained as a reflection of the DON induced decrease in total IgG concentration. Tryphonas et al. (1986) showed that certain blood proteins were non-specifically decreased in animals consuming DON, and one could speculate that IgG was similarly reduced. Again these observations may also be a secondary effect of DON-induced feed refusal.

The observation that DON feeding increased <u>in vitro</u> IgA production by splenocytes was important because it favors the possibility that DON affected synthesis of IgA rather than catabolism. A large amount of IgA produced is catabilized by the liver. Vaerman et al., (1978) proposed that in rodents, the liver functions as an "IgA pump" that regulates serum levels of IgA by transporting circulating polymeric IgA into the bile. Thus blockage of catabolism might be explained to cause elevated serum IgA. However the observation that spleen cell cultures from DON treated mice produced elevated IgA favors the possibility that DON was affecting the

regulation of IgA synthesis.

Mitogen stimulated splenic lymphocytes from mice fed dietary DON produced much larger amounts of IgA in vitro than their respective restricted and ad lib controls (Fig. 6). The total IgA produced in the DON groups relative to its controls was larger in the spontaneous than in the LPS stimulated group. LPS is a polyclonal B cell mitogen. One would expect LPS-stimulated B cells to produce larger amounts of immunoglobulin than in non-mitogen stimulated (spontaneous) controls. It is possible splenic IgA lymphocytes from DON treated animals were already stimulated and did not require mitogen to stimulate B cells to produce immunoglobulin. This could be argued by observations in the Con A and Con A-LPS mitogen stimulated IgA cultures. Elson et al., (1979) demonstrated that Con A-pulsed spleen T cells added to fresh LPS-stimulated B cell cultures induced the suppression of IgA, IgG, and IgM synthesis. The authors point out that this suppression is mediated by T cells. Fig. 6 shows that IgA produced in Con A-LPS stimulated cultures from DON treated animals were similarly less than that produced in LPS-stimulated cultures from these same animals. The suppression exhibited by Con A alone in these cultures further argues for prior stimulation of lymphocytes in DON treated animals. Although the preparation of spleen cell cultures in this thesis did not involve the separation of B and T cells as was done by Elson et al., the results of the in vitro studies using crude splenocyte cultures support the observation of Elson et al. and suggests prior stimulation of

splenic lymphocytes from DON treated animals. A further argument for lymphocyte stimulation by DON can be made with the antigen-specific response data. Non-immunized mice fed dietary DON exhibited an increased cholera toxin-specific equivalent IgA level (Fig 4). This suggests that dietary DON by causing an increased IgA response in a non-immunized host might be acting as a polyclonal stimulator of IgA production.

There was no significant difference in IgG production in cultures between DON-treated or control mice. In contrast to IgA, there is very little IgG produced in the non-mitogen stimulated cultures from DON treated animals. This was also true for IgM production from spontaneous cultures of DON treated animals. There was a trend toward decreased IgM production in mitogen stimulated cultures from DON treated animals but these results were significant in only one of three experimental trials (table 13). In general these results support the concept of isotype-specific dysregulation of immunoglobulin production caused by DON.

The major success of the research in this thesis was the development of a model for studying trichothecene-induced dysregulation of IgA synthesis. Since much of the information is preliminary it is not possible to make specific conculusions on the mechanistic basis for this effect. However it is possible to speculate as to possible causes for the observations and suggest further experiments. First, the possibility of increased permeation of the dietary protein casein eliciting an IgA immune response was addressed earlier in this thesis. Future experiments should further

investigate antigen-specific IgA production in isolated splenic lymphocytes from the spleen and Peyer's patches. This would help to determine if the DON effect represents an antigen specific response rather than a polyclonal effect. A second potential explanation for the increase in serum IgA would be an increase in the number of IgA producing cells. There are many potential ways that this could occur. One would be through a direct effect of DON. Recently Miller and Atkinson (1986) demonstrated that low DON concentrations induced the release of the lymphokine interleukin 1 (IL-1) from peritoneal macrophage. IL-1 release from antigen presenting cells such as macrophage could initiate a cascade of T cell activity involving the further release of lymphokines to mediate immune activity. Murray et al., (1987) demonstrated that the lymphokine IL-5 produced by T cells causes secretion of IgA by B cells and that this secretion is magnified two to threefold in combination with IL-4. IL-4 and IL-5 are produced by the T helper 2 (Th_2) subset of T cells. Gamma interferon is produced by the Th_1 subset of T cells and has been shown to down-regulate IL-5 stimulation of IgA secretion. The authors suggest that a higher proportion of Th_2 relative to Th_1 cells reside in the Peyer's patches. The effects of trichothecenes on lymphocyte stimulation suggest that DON may be affecting T cells and accessory cells such as macrophage. This could ultimately involve interleukins at the Peyer's patch level which could theoretically alter IgA regulation. Future experiments should examine spleen and Peyer's patch cultures from DON

treated animals for an increase in interleukin activity, specifically IL-4 and IL-5.

In summary, the data presented here demonstrate that exposure to dietary DON increases serum IgA and concurrently decreases IgG and IgM. The antigen-specific immunoglobulin response to cholera toxin and casein reflects the total immunoglobulin profile generated upon dietary DON exposure. Splenocytes from mice fed dietary DON produced increased IgA in mitogen stimulated and spontaneous cultures. These results suggest that dietary DON alters the regulation of IgA production.

The dysregulation of IgA production has been associated with a common form of glomerulonephritis known as IgA nephropathy. This nephropathy is characterized by accumulation of IgA in the mesangial region of the kidney glomerulus. IgA has also been shown to inhibit <u>in vitro</u> natural killer cell activity which attack tumor, virus-infected, and undifferentiated normal cells. This research demonstrated a dysregulation of IgA production in the systemic compartment be dietary DON and provides a model for further study of the effects of mycotoxins on the immune system.

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