BRIEF REPORT

DOSE-DEPENDENT INDUCTION OF ANTI–NATIVE DNA ANTIBODIES IN CATS BY PROPYLTHIOURACIL

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Cats receiving propylthiouracil (PTU) develop antinuclear antibodies (ANA) and an immune-mediated disease syndrome characterized by anorexia, lymphadenopathy, weight loss, and Coombs-positive hemolytic anemia. Investigation of the ANA specificity indicated that the predominant ANA activity consisted of anti–native DNA (nDNA) antibodies. The formation of anti-nDNA antibodies and immune-mediated disease syndrome appeared to be dose-dependent, even in cats in which a response had been induced on 4 prior occasions. These results supply further evidence that PTU-induced autoimmunity is not the result of a simple drug allergy. Rather, it appears that PTU induces a lupus-like syndrome, including the hallmark sign of systemic lupus erythematosus, anti-nDNA antibodies.

More than 25 drugs have been implicated as causes of antinuclear antibodies (ANA) and drug-induced lupus-like syndromes in humans (1,2). Studies of drug-induced ANA formation have relied on human subjects and have therefore been limited. Until recently, there has been no animal model of drug-induced ANA and immune-mediated disease (IMD) in which to examine the mechanism for autoantibody formation and its significance in disease pathogenesis. Previously, we reported a unique animal model of an IMD syndrome that occurred in more than 50% of mongrel cats receiving 150 mg/day of the antithyroid drug, propylthiouracil (PTU) (3–5). This syndrome is characterized by anorexia, lymphadenopathy, weight loss, and hemolytic anemia, with autoantibodies directed against red blood cells and nuclear components (ANA).

Several autoantibodies recognizing nuclear, cytoplasmic, or cell membrane structures have been characterized in human patients with the prototypic IMD, systemic lupus erythematosus (SLE), as well as in patients with drug-induced lupus (6,7). Anti–native DNA (nDNA) antibodies have received considerable attention, because in high titers, they are considered specific for idiopathic SLE (6,8). They are also involved in the development of the pathology of tissue injury seen in SLE patients (9). The role of other autoantibodies in the development of disease is less clear.

The mechanism of formation of autoantibodies in SLE patients is unknown; however, for drug-induced IMD, including drug-induced lupus, a number of theories have been proposed. First, the drug may act as a hapten, binding to self-determinants that render the complex immunogenic. Alternatively, the drug may denature self-components, exposing or creating foreign epitopes, thereby stimulating antibody production. Last, the drug could be acting directly on specific immunoregulatory cells, altering self-tolerance (1,2,10).

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Preliminary reports on PTU-induced IMD in cats have indicated that induction of this disease requires a free sulfhydryl group on PTU (3,5). The present report expands on this finding by characterizing the ANA specificity and demonstrating the dose-dependent nature of PTU-induced IMD. Our findings are consistent with the hypothesis that PTU or its metabolite(s) disrupts immune tolerance and leads to the development of a lupus-like disease.

MATERIALS AND METHODS

Animals. Cats free of specific pathogens were obtained from the Feline Research Center (Ithaca, NY). The characteristics of these cats and the disease-induction protocol have been described previously (3). Briefly, 17 domestic short-hair cats of both sexes were given capsules of PTU orally, 150 mg/day, for 8 weeks. Appetite, activity, and appearance were evaluated daily. Blood was drawn weekly for a complete blood count, direct Coombs' test, and ANA determination. PTU was discontinued prior to completion of the full 8 weeks in cats that developed the disease syndrome. Eight cats developed ANA and the disease syndrome within 4.5 ± 0.6 weeks (mean ± SEM).

Anti-DNA and antihistone antibody assays. Sera from 6 of the 8 cats with PTU-induced IMD syndrome and ANA titers >1:40 were examined for anti-nDNA and anti–denatured (single-stranded) DNA (dDNA) activity using a solid-phase enzyme-linked immunosorbent assay (ELISA) (11). The highly purified nDNA substrate (Behring Diagnostics, La Jolla, CA) was treated with S1 nuclease prior to use in the assay, to prevent contamination with single-stranded (denatured) regions. Antihistone antibodies were determined by ELISA using total calf thymus histones (Behring Diagnostics) containing all 5 histone fractions (11,12).

***Crithidia luciliae* and ANA assays.** Anti-nDNA antibodies were confirmed by a less sensitive, but essentially unambiguous, indirect immunofluorescence (IF) assay, using the kinetoplast of *C luciliae* as an nDNA substrate (Kallestadt, Austin, TX) (13). ANA was examined by indirect IF using HEp-2 cells as a nuclear substrate and affinity-purified fluorescein isothiocyanate (FITC)–labeled anti-cat IgG (heavy and light chain–specific; Pel-Freez, Rogers, AR).

Chromatin absorption studies. To evaluate the contribution of anti-nDNA antibodies to the total ANA activity, 4 serum samples were absorbed with chromatin, a natural nDNA-containing complex isolated from calf thymus cell nuclei, as previously described (14). Serum (7 μl) from 4 cats with anti-nDNA antibodies was diluted into 350 μl of phosphate buffered saline, and added to 1 mg of calf thymus chromatin that had been precipitated in a microfuge tube. After agitation for 2 hours at 4°C, the chromatin was pelleted by centrifugation, and the supernatant was assayed for reactivity on HEp-2 cells by indirect IF using FITC-labeled rabbit anti-cat IgG (heavy and light chain–specific; Pel-Freez). Another aliquot of the supernatant was diluted 1:4 and assayed by ELISA for anti-nDNA antibodies.

HCl extraction of rat liver sections. Acid extraction of rat liver sections removes histones and most of the nonhistone nuclear protein from the cell nuclei, leaving DNA as the predominant nuclear component of the cells (15). Therefore, ANA determinations were performed by IF using frozen rat liver sections as the substrate (BioDx, Denville, NJ), before and after incubation in 0.1*N* HCl, to determine the contribution of non-nDNA antibodies to the overall ANA activity.

Effect of PTU dose in previously responding cats. Earlier studies showed that after discontinuation of PTU, the ANA and IMD syndrome resolved within 1–4 weeks (mean ± SEM 1.9 ± 0.4 weeks). Cats that had been given 2 or more courses of 150 mg/day of PTU were challenged with a smaller (12.5 mg/day) dose, to determine if induction of these anti-nDNA antibodies and IMD syndrome was due to a dose-dependent action of PTU. Four of the 8 responding cats (3 females, 1 male) were used in this study. Of these, 3 had developed ANA and IMD syndrome on 2 prior occasions, and 1 had developed ANA and IMD syndrome 4 times, following challenges of orally administered PTU, 150 mg/day (3). The cats had not received PTU for 3 months and had been free of clinical disease and autoantibodies for 2 months. Each cat received an oral dose of PTU (Eli Lilly, Indianapolis, IN), 12.5 mg/day for 8 weeks. Cats were examined daily for appetite, activity, lymphadenopathy, and pallor, and blood was drawn weekly by jugular venipuncture for direct Coomb's test and ANA determinations.

RESULTS

PTU-induced ANA specificity. All cats with ANA titers >1:40 had significant anti-nDNA activity, and 5 of the 6 were positive by IF using *C luciliae* (Table 1). All cats were anti-dDNA positive as well (data not shown). Because denatured DNA preparations contain portions of native helical structures (16), it could not be determined readily if anti-dDNA antibodies were another, separate population of PTU-induced antibodies; how-

Table 1. Anti–native DNA (nDNA) and antihistone activity of propylthiouracil (PTU)–induced antinuclear antibodies (ANA)*

Cat no.	IF (titer)		ELISA (OD)	
	ANA	*Crithidia luciliae*	nDNA	Total histones
974	1:640	1:40	2.67	0.02
973	1:640	1:40	2.90	0.07
632	1:640	<1:10	1.50	0.03
975	1:640	1:40	2.85	0.01
643	1:640	1:80	4.50	0.19
656	1:640	1:20	2.45	0.05
NCS	<1:40	<1:10	0.15 ± 0.12 (0–0.40)	0.04 ± 0.06 (0–0.16)

* The ANA titer was determined by immunofluorescence (IF) on HEp-2 cells and kinetoplast titer on *C luciliae*, using a fluorescein isothiocyanate–conjugated anti-cat IgG (heavy and light chain–specific). For the enzyme-linked immunosorbent assay (ELISA), sera were diluted 1:200, and antibody binding was detected with a peroxidase-conjugated anti-cat IgG (heavy and light chain–specific). Negative control sera (NCS) were drawn from 9 of the study cats prior to administration of PTU. Values for NCS are the mean ± SD (2 SD range). OD = optical density.

ever, since anti-dDNA antibodies do not contribute to the ANA activity, their presence was not germane to the analysis of ANA specificity. Control specimens for these assays were drawn from the same cats prior to PTU treatment. The level of PTU-induced anti-nDNA activity was 10–20-fold above the level in these controls, and was comparable with the anti-nDNA activity observed in SLE patients (11,12). ELISA findings revealed no antihistone activity in any of the serum samples tested (Table 1).

Contribution of anti-nDNA antibodies to ANA activity. Following absorption with chromatin, the anti-nDNA antibodies (detected by ELISA) and virtually all the ANA activity were removed from 3 of 4 cats, and were substantially reduced in the other (Table 2). Significantly, there was no apparent change in cytoplasmic IF staining at 1:50 dilution of the cats' sera (Figure 1), which indicates that antibodies to cytoplasmic constituents may also exist, but constitute a separate antibody population.

ANA titers were unchanged or higher following acid extraction of the rat liver substrate, and the IF staining was more intense. These findings further support the conclusion that anti-nDNA antibodies accounted for the bulk of the ANA activity.

Effect of dose on disease induction. The mean ± SEM time for reinduction of clinical disease in cats receiving 150 mg/day of PTU was 2.6 ± 0.8 weeks (3). In contrast, during retreatment with PTU at 12.5 mg/day, all cats remained clinically and serologically normal throughout the 8-week study. To insure that these cats were still capable of developing PTU-induced IMD, they were given the standard induction dosage of 150 mg/day of PTU at 1 month following the completion of the study using the lower dose. All 4 cats developed the IMD syndrome within 3 weeks.

DISCUSSION

These findings and those of previous studies (3) support our contention that PTU-induced IMD syndrome in cats is not a simple drug allergy. It is unlikely that PTU is acting merely as a hapten or is modifying self-antigens, since these mechanisms for drug allergy would not be expected to require high doses of PTU following previous episodes of drug-induced IMD (17)

Table 2. Contribution of anti-nDNA antibodies to total ANA activity*

Cat no.	Unabsorbed			Chromatin absorbed		
	IF		ELISA (OD)	IF		ELISA (OD)
	Nuclear	Cytoplasmic	Anti-nDNA	Nuclear	Cytoplasmic	Anti-nDNA
974	3+	2+	1.25	1+	2+	0.03
973	1+	3+	5.75	–	2+	0.44
656	3+	2+	5.31	2+	2+	0.14
643	4+	3+	8.91	–	2+	1.53
Id12†	4+	–	9.14	–	–	0.02

* ANA determinations performed on HEp-2 cells were graded for staining location and intensity, on a scale of 1+ to 4+. The cat sera were absorbed with chromatin (see Materials and Methods) and the supernatant assayed for reactivity on HEp-2 cells by IF and for anti-nDNA by ELISA. – = negative; see Table 1 for other definitions.

† Id12 is a mouse monoclonal anti-nDNA antibody previously described (ref. 20) and used here as a positive control. Not shown is the effect of chromatin absorption on human sera containing anti-tetanus activity. Following absorption, no alteration in antibody activity occurred, demonstrating that chromatin did not nonspecifically bind immunoglobulin.

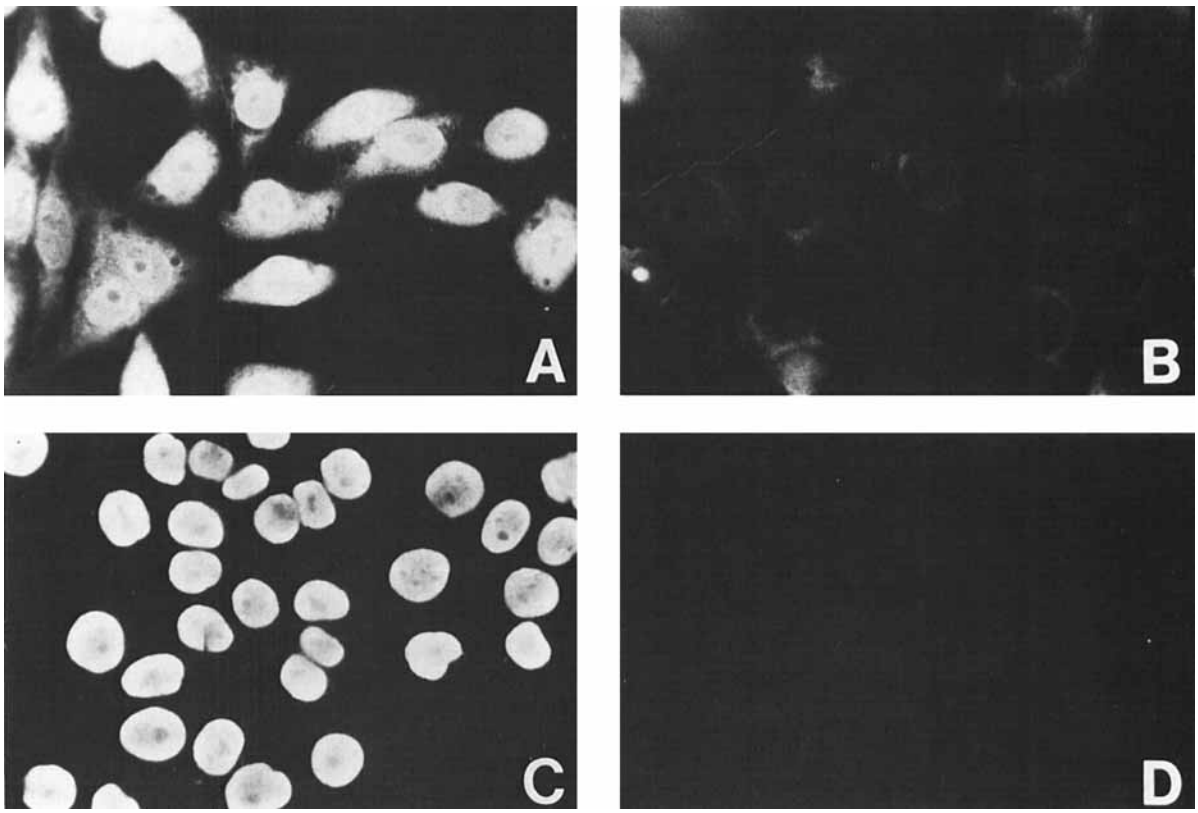

Figure 1. Immunofluorescence staining of HEp-2 cells from cat no. 643 (**A, B**) and mouse monoclonal anti-nDNA antibody (1d12; see ref. 20) (**C, D**). **A** and **C**, Before absorption with chromatin. **B** and **D**, After absorption with chromatin. (Magnification × 400.)

(i.e., induction of autoantibodies by PTU lacks immunologic memory). Induction of disease required more than 12.5 mg/day of PTU for 8 weeks, even in a cat that had an induced response on 4 previous occasions.

The specificity of the elicited ANA was determined in order to identify the target autoantigen and examine possible mechanisms underlying autoantibody induction. The detection of anti-nDNA antibody in cats with PTU-induced IMD syndrome is highly significant, because this antibody is a hallmark of idiopathic SLE and is rarely reported in drug-induced lupus syndromes in humans (1,2,6,8). It is unlikely that a mechanism involving modification of DNA by PTU would be the explanation for the formation of this antibody, because an altered DNA would not be expected to induce antibodies to epitopes within the native molecule (8,10). The previously reported inability to detect anti-nDNA antibodies (3) appears to have been due to lack of adequate controls in the earlier immunoassay for cat immunoglobulin. Since the induction of these antibodies was unexpected and is an unusual result of any drug-induced IMD syndrome, we carefully confirmed the presence of these antibodies in this study by using 2 different assays. The Farr assay was not used in our study because cats, like many mammals, have a serum DNA binding protein that precipitates the radiolabeled DNA and thus produces erroneous results (18). Rather, we chose an ELISA, which has been validated for both the murine and human antibodies (11,12), to test for nDNA antibodies. Additionally, we used the *C luciliae* IF assay which, although less sensitive than the ELISA, is essentially an unambiguous assay for anti-nDNA antibodies (13).

Chromatin was used as the immunoabsorbent because it is an abundant source of nDNA, containing little, if any, denatured DNA regions. Additionally, sera could be treated with large quantities of antigen, followed by physical removal of bound antibodies, due to the insolubility of chromatin in physiologic solutions. It could be argued that absorption with chroma-

tin was due to binding of antibodies to non-DNA components. By ELISA, however, we could not demonstrate antibodies to histones, the predominant protein constituent of chromatin. Furthermore, there was no reduction in the ANA titers following acid extraction of the liver sections, a procedure that removes histones and the bulk of nonhistone protein components from the nucleus (15). The results of this assay corroborate the lack of antihistone activity indicated by the ELISA. Additionally, they substantiate the interpretation of the chromatin absorption studies indicating that the majority of ANA activity was due to anti-nDNA antibodies.

Although anti-nDNA antibodies are predominant, other autoantibodies are formed during the course of disease. Anti–red blood cell antibodies have been detected, and there is preliminary evidence from cytoplasmic immunofluorescence and immunoprecipitation experiments (3,5) that antibodies to other self-components are likely to exist.

The importance of this animal model is twofold. First, it is unique in its induction of anti-nDNA antibodies as the predominant immune response. These antibodies are sufficiently unusual to be one of the criteria for the diagnosis of SLE in humans (19). Because anti-nDNA antibodies are not inducible by classic immunization procedures, however, the study of these autoantibodies has been restricted to those from SLE patients or from mice with lupus. Second, this model may prove useful to investigations of the mechanism of abnormal immune regulation leading to autoantibody formation and the role these antibodies play in the induction of clinical disease.

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