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Androgen control of immunocompetence in the male house finch, *Carpodacus mexicanus* Müller

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Summary

The immunocompetence handicap (ICH) hypothesis predicts that elevated levels of the gonadal androgen testosterone (T) entail obligatory costs, such as immunosuppression, but evidence supporting this immunosuppressive influence is equivocal. To investigate this question, adult males house finches, *Carpodacus mexicanus*, were exposed to short days and chronically treated with T-filled (T males; *N*=10) or empty (C males; *N*=10) Silastic capsules. Testosterone administration increased plasma T levels and the size of the cloacal protuberance, an androgen-dependent secondary sexual characteristic. To study humoral immunity, finches received injections of sheep red blood cells (SRBC) and we measured circulating concentrations of antibodies to these cells with a hemagglutination test. All males produced antibodies following four SRBC injections at weekly intervals. Antibody titers in T and C males did not differ 5 days after the fourth injection, but were 59% lower in T than C males 2 weeks later. To study cell-mediated immunity, we measured the local inflammatory response to an injection of phytohemaglutinin (PHA). This response in T and C males was similar 1 day after PHA injection, but was 58% less in T than C males 2 days following the injection. Thus, T and C males mounted similar humoral and cell-mediated immune responses, but T treatment compromised maintenance of these responses. The results, demonstrating immunosuppressive effects of elevated T, are consistent with the ICH hypothesis.

Key words: androgen, cell-mediated immunity, humoral immunity, immunocompetence handicap hypothesis, inflammation.

Introduction

Behavioral ecologists, sociobiologists and physiologists are seeking to understand how reproductive hormones affect sexual secondary characteristics, the immune system and male quality (Sheldon and Verhulst, 1996). According to one hypothesis [immunocompetence handicap (ICH) hypothesis: Folstad and Karter, 1992] sexually selected male characteristics reliably indicate male quality. Elevated circulating concentrations of the testicular androgen, testosterone (T), promote the development and maintenance of these characteristics, but also suppress certain physiological parameters and, in particular, may be immunosuppressive. Consequently, all males of a population may potentially respond to elevated T levels by developing sexually selected characteristics. However, 'low status' males, i.e. males that cannot concurrently withstand this development and the immunosuppressive influence of T, are at a disadvantage because they suffer higher susceptibility than 'high-status' males to diseases and parasitic infections. Alternatively, 'low status' males, instead of jeopardizing their immune responses, may maintain lower plasma testosterone and less developed sexual ornaments than 'high status' males, resulting in less success in mate choice.

Gonadal hormones exert multiple and complex influences on immune functions (review: Ahmed and Talal, 1990). Lymphocytes in some species of fish have androgen receptors (Slater et al., 1995) and consistent with the ICH hypothesis, T in mammals and fish is generally immunosuppressive (Ahmed et al., 1987; Angele et al., 1998; Benten et al., 1993; Slater and Schreck, 1997; but see Bilbo and Nelson, 2001). By contrast, evidence for immunosuppressive effects of T in birds, which have been used extensively to test the ICH hypothesis (e.g. Lindström et al., 2001; Norris et al., 1994; Saino and Møller, 1994; Seutin, 1994; Zuk and Johnsen, 1998), remains equivocal. Testosterone treatment suppressed humoral and cell-mediated immunity in European starlings, *Sturnus vulgaris* (Duffy et al., 2000) and dark-eyed juncos, *Junco hyemalis* (Casto et al., 2001). This treatment was also immunosuppressive in house sparrows, *Passer domesticus* (Evans et al., 2000), song sparrows, *Melospiza melodia* (Owen-Ashley et al., 2004) and captive superb fairy-wrens, *Malurus cyaneus* (Peters, 2000). Given to other species, however, T had no detectable influence on immune responses (red-winged blackbirds, *Agelaius phoeniceus*: Hasselquist et al., 1999; greenfinch, *Carduelis chloris*: Lindström et al., 2001).

Several factors may account for these differences. For example, the above studies investigated various aspects of the immune system (antibody production; local inflammatory response; number of circulating lymphocytes; Nava et al., 2001) that may have different latencies to respond and different sensitivities to T. Additionally, heterologous antibody production, which is often used to estimate humoral immunity, depends on the reproductive stage (Nelson et al., 1998; Von Schantz et al., 1999). This production was measured at various times ranging from 5 to 13 days after antigen administration (Evans et al., 2000; Casto et al., 2001; Peters, 2000), but not all these studies examined the time course of antibody production, in which cases antibody production may not have been measured at its peak. Furthermore, the immunization protocol used in the above studies often failed to induce detectable antibody concentrations in all experimental subjects. In such cases, data for non-responsive individuals either were retained in (Evans et al., 2000; Peters, 2000) or were excluded from (Casto et al., 2001) statistical analyses. Finally, effects of T on immunity may be indirect and mediated by complex and poorly understood interactions between this and other hormones, in particular corticosterone (CORT) as well as by changes in body energy allocation (Evans et al., 2000; Owen-Ashley et al., 2004). If indirect, these effects likely vary as a function of physiological parameters other than the reproductive status.

To address the above issues, we researched the influence of T treatment concurrently on three rather than single aspects of the immune system: cell-mediated immunity, humoral immunity and lymphocyte numbers. This research was done on adult male house finches, *Carpodacus mexicanus*, a species that has been used extensively to investigate the bases of mate selection (Hill, 1990, 1991), the mechanisms that control a sexually selected trait (plumage coloration: Hill et al., 1994), correlations between parasite infections and this trait (Thompson et al., 1997), the bases of disease resistance (Duckworth et al., 2004) and the environmental control of reproductive physiology (Hamner, 1968). Moreover, T plays a role in sexual signaling in the house finch, being positively correlated with its most important sexual signal, plumage redness (Duckworth et al., 2004) and T treatment to this species exacerbates coccidian infection (Duckworth et al., 2001). Taken together, these studies suggest a role for T in maintaining honesty of the plumage color through effects on immune responsiveness. To increase the likelihood of birds producing measurable levels of antibody in response to immunization, each subject received multiple rather than a single antigen injections. In addition, we determined plasma antibody concentrations in response to T administration several times during and after immunization (Von Schantz et al., 1999; Peters, 2000). Finally, extraneous factors that potentially interact with the effects of the experimental treatment were standardized by holding birds in identical conditions and in a strictly controlled environment for the duration of the study.

Materials and methods

Bird capture and maintenance

Twenty adult male house finches (*Carpodacus mexicanus* Müller) were caught using seed-baited Potter traps in Tempe, Arizona, 31 January to 2 February 2001. We brought birds into captivity, marked them with a numbered aluminum leg band to permit individual identification and placed them in individual cages in a lightproof environmental chamber at constant ambient temperature for the duration of the study. We exposed birds to short day length (8 h:16 h L:D; lights on at 07:00 h) to prevent the development of their reproductive system. Finches received food (Mazuri finch chow) and water *ad libitum*.

Testosterone capsule implantation and removal

On February 16, we randomly divided males into two groups. Ten males (T males) received two subcutaneous T-filled Silastic capsules prepared as described in Dloniak and Deviche (2001) and 10 males (C males) received two identical, but empty capsules. Each T-filled capsule was 11 mm-long and the actual portion filled with hormone approximated 9 mm. Capsules were inserted under the skin through a 2 mm-long incision along one flank. In previous studies, a similar T treatment increased plasma T concentrations in house finches (Duckworth et al., 2004; Stoehr and Hill, 2001) and other avian species of similar sizes (dark-eyed junco: Ketterson and Nolan, 1992) and it maintained physiologically high circulating steroid concentrations that were close to those measured at the beginning of the breeding season for up to several months. We defined the day that capsules were administered as Day 0 (D0) and all data are reported with reference to this date (Table 1). We weighed capsules before implantation and again after removal (D66: five T males; D74: five remaining T males) and oven drying at 37°C for several days and used the difference between the initial and final weights of the capsules to calculate that the average release rate of T was 112±2 μg day^{-1} bird^{-1} (mean ± S.D.). This rate compares favorably to that in another passerine of similar size (dark-eyed junco: 92 μg day^{-1} bird^{-1}; Deviche, 1992).

Blood samples

We collected up to 350 μl of blood (<2% body mass) from a wing vein of each finch into heparinized microhematocrit tubes on D-3, D10, D20, D39 and D55. We used approximately 5 μl of blood to prepare thin smears on glass microscope slides (Bennett, 1970; Deviche et al., 2001a) and kept the remainder of each sample on ice until it was centrifuged at 4°C within 2 h of collection. Plasma was harvested and stored at –20°C until assayed for T and antibody concentration.

Testosterone assay

We measured total circulating plasma T concentrations using a commercial competitive immunoassay (ELISA; AssayDesigns, Inc., Ann Arbor, MI, USA). All samples (5 μl plasma per assay well) were assayed simultaneously, in duplicate and in a random order and according to the

Table 1. *Summary of experimental manipulations*

	D-3	D0	D10	D13	D20	D27	D34	D39	D55	D63	D64	D65	D66
Silastic capsule implantation		×											
Sheep red blood cell injection				×	×	×	×						
PHA injection									× (priming)	×			
Blood collection	×		×		×			×	×				
Body mass and CP width	×		×		×			×	×				
Wing web thickness determination										×	×	×	×

All data are reported with reference to the day that birds received Silastic capsules (Day 0=D0). See text for further details on experimental procedures.

manufacturer's specifications. Each ELISA plate included a complete standard curve and positive and negative controls. We calculated plasma T concentrations using Prism version 3.02 (Graphpad Software, Inc., San Diego, CA, USA). Validation tests demonstrated that the assay measures T in house finch plasma accurately and reliably (Results).

Morphological parameters

Immediately after blood collection, we weighed birds to the nearest 0.1 g and measured their cloacal protuberance width (an androgen-dependent secondary sexual characteristic: Deviche, 1992; Dloniak and Deviche, 2001) to the nearest 0.1 mm using digital calipers.

Immunization and humoral immunity

We immunized finches against freshly (i.e. same day) washed sheep red blood cells (SRBC; Hemostat, Dixon, CA, USA) using a modification of the Casto et al. (2001) protocol. For this, each male received a weekly 0.15 ml i.p. injection of 2% SRBC (D13, D20, D27 and D34). Injections on D20 were given after collection of blood samples and morphological data. For the first injection (D13) washed SRBC were suspended in 0.15 ml Freund's Complete Adjuvant (ICN Biomedicals, Inc., Aurora, IL, USA). Cells used for subsequent injections (D20, D27 and D34) were suspended in 0.15 ml of 0.1 mol l^{-1} phosphate buffer. We found no evidence for detrimental effects of the complete adjuvant injection as measured by changes in body mass (see Results), signs of distress or inflammation (P.D., personal observations) or mortality. We measured the plasma concentration of antibodies to SRBC with an *in vitro* hemagglutination test (Casto et al., 2001), using a commercial antiserum against SRBC (ICN Biomedicals, Inc., Aurora, IL, USA). For this assay, plasma was diluted serially in 0.1 mol l^{-1} phosphate buffered saline (PBS) and all samples were assayed in duplicate and in a random order. We collected data without knowledge of the sample identity and expressed results as the number of wells showing hemagglutination. Each assay plate included negative and positive controls to ensure standardization.

Cell-mediated immunity

We assessed cell-mediated immunity by measuring the local inflammatory response to a unilateral injection of the mitogenic plant protein phytohemaglutinin (PHA; Sigma Chemical Co., St Louis, MO, USA; Casto et al., 2001; Lochmiller et al., 1993). Birds received a priming s.c. injection of PHA (0.25 mg in 50 µl PBS) into the left wing web on D55 and a second, identical injection on D63. Wing web thickness (mean of three consecutive determinations) was measured to the nearest 25 µm with a pressure-sensitive dial thickness gauge (Mitutoyo, model 7226) immediately before the D63 challenge injection and 24 h (D64), 48 h (D65) and 72 h (D66) later. Results are presented as the change in web thickness relative to D63.

The Arizona State University Institutional Animal Use and Care Committee approved all experimental protocols and birds were caught under current federal and Arizona state scientific collecting permits.

Lymphocyte numbers

We stained blood smears using the May-Grunwald and Giemsa technique. Stained smears were air-dried, dehydrated overnight under vacuum, cleared with xylene and coverslipped using Cytoseal 60 (VWR). We studied smears with a light microscope and digitized 20 randomly selected visual fields of each smear at 400× magnification with a color digital camera. Using Image Pro (Media Cybernetics, Silver Spring, MD, USA), we automatically counted the number of erythrocytes in each digitized image and manually counted the number of lymphocytes in the same image. The average number of erythrocytes counted per smear was 10,587±1290 (mean ± S.D.). We present data as numbers of lymphocytes counted per 1000 erythrocytes.

Statistical analyses

Unless otherwise specified, we analyzed differences between C and T males as a function of time using two-way analyses of variance for repeated measures (2RANOVA) and, when appropriate, Student–Newman–Keuls multiple pairwise comparison tests (StatSoft Statistica, version 5.1, Statsoft Inc., Tulsa, OK, USA). Data sets not complying with normality and/or homoscedasticity requirements for 2RANOVA were ranked prior to analysis (Conover and Iman, 1981). Results from analyses of untransformed data are presented as mean ± S.D. and results from analyses of ranked data are shown as medians ±0.5 interquartile intervals (Results and figure

legends). We compared the slopes of a T assay standard curve and a curve generated by serially diluting house finch plasma using Graphpad Prism. The significance level of all statistical tests was set at α=0.05.

Results

Plasma testosterone concentrations

Assay validation

The monoclonal antibody used in the T ELISA has negligible (<0.5%) cross reactivity with dihydrotestosterone, estradiol, progesterone, corticosterone and dehydroepiandrosterone (manufacturer's specifications). The intraassay coefficient of variation was 8.13% (median) and the assay sensitivity was 5.4 pg ml^{-1} plasma. A curve generated by assaying finch plasma at various dilutions was parallel to a standard curve (Fig. 1; Hill slopes: finch plasma dilution curve: –0.7544±0.1464 (S.E.M.); standard curve: –0.7538±0.1276 (i.d.); P>0.05). We added T at concentrations between 0.125 ng ml^{-1} and 2 ng ml^{-1} to plasma obtained from C males and found the concentrations of T measured to correlate to those added (linear regression line: slope=0.66; intercept: 0.018 ng ml^{-1}; coefficient of determination: r^2=0.996). Finally, we compared T concentrations in plasma obtained from T males (N=3) and from which steroids had or not been eliminated with dextran-activated charcoal (Wingfield et al., 1984). Treatment of plasma samples with dextran-activated charcoal before the assay reduced T concentrations in these samples by more than 97%. The assay, therefore, measures house finch plasma T specifically.

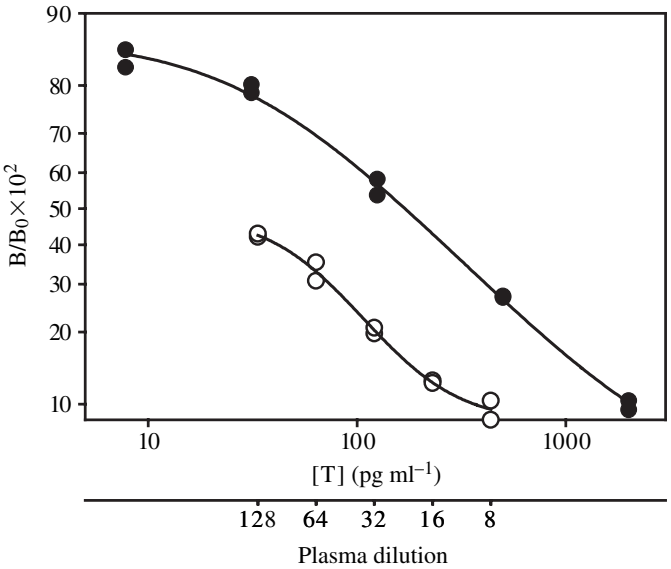

Fig. 1. Standard curve of the ELISA assay used to measure plasma testosterone (T) concentrations (filled circles). The curve represents the percentage T bound to the antibody ($B/B_0\times10^2$) as a function of the T concentration added to the assay well. The graph also shows the curve obtained by assaying T in a house finch plasma sample at various dilutions (open circles). Each point represents one assay well.

Group differences

Plasma T levels varied as a function of time (upper panel, Fig. 2; $F_{4,68}$=28.2, P<0.0001) and were influenced by the experimental treatment ($F_{1,17}$=115.5, P<0.0001). There also was a significant time × treatment interaction ($F_{4,68}$=13.1, P<0.0001). Before capsule implantation plasma T levels were low and similar in C and T males. Testosterone levels in C males remained low (less than 0.5 ng ml^{-1}) throughout the study period. They were higher on D39 (0.41±0.20 ng ml^{-1}) and D55 (0.38±0.17 ng ml^{-1}) than on D-3 (0.18±0.02 ng ml^{-1}), D10 (0.29±0.12 ng ml^{-1}) and D20 (0.22±0.16 ng ml^{-1}; Student–Newman–Keuls tests: P<0.05), but remained within the limits of levels measured outside the reproductive season

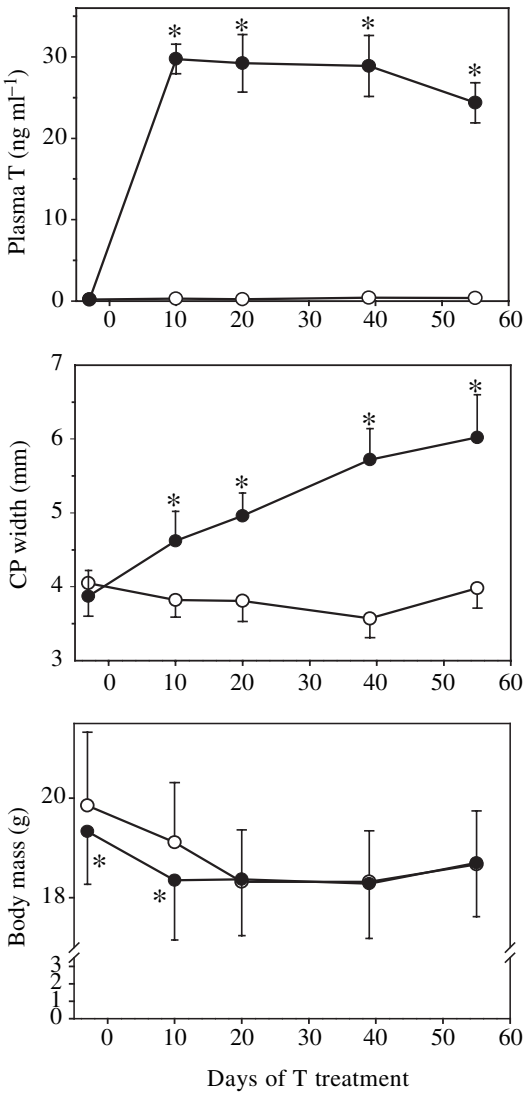

Fig. 2. Plasma testosterone (T) concentrations (median ±0.5 interquartile intervals), widths of the cloacal protuberance (CP; id.) and body masses (mean ± S.D.) of adult male house finches as a function of time after the beginning of T administration on Day 0. *Indicates a significant difference (Student–Newman–Keuls test, P<0.05) between T-treated (filled circles; N=10) and control (open circles; N=10) males.

in non-breeding males (personal observations). The small increase (on average, 0.23 ng ml^{-1}) in plasma T in C males during the course of the study may reflect slow maturation of the reproductive system despite short day exposure, which may itself have resulted from C males hearing singing T males. Testosterone levels after capsule administration were evenly higher in T than C males. This difference persisted for the duration of the study.

External morphology

Cloacal protuberance widths changed as a function of time (middle panel, Fig. 2; $F_{4,72}$=9.6, P<0.0001) and were affected by T administration ($F_{1,18}$=27.9, P<0.0001) and there was a time × treatment interaction ($F_{4,72}$=23.3, P<0.0001). On D-3 CPs in both groups were small and undeveloped. They remained undeveloped in C males throughout the study but gradually enlarged in T males, so that the two groups differed starting on D10.

Body masses did altogether not differ between C and T males, but changed ($F_{4,72}$=24.8; P<0.0001) in a group-dependent manner (lower panel, Fig. 2; group × time interaction: $F_{4,72}$=3.15, P<0.02) during the course of the study. Testosterone-treated males had lower body masses than C males on D-3 and D10, but not at later sampling times.

Humoral immunity

We detected no antibody to SRBC in the plasma of any male prior to (D-3 and D10) or 1 week after (D20) the first SRBC injection. However, all samples collected 5 days after the fourth antigen injection (D39) and 80% of the samples collected 2 weeks after this injection (D55), contained measurable antibody concentrations. Concentrations on D39 and D55 were correlated (Pearson product moment correlation: r=0.657, P<0.002; N=20). Due to the general absence of detectable antibody before D39, we compared groups of data with ANOVA only for D39 and D55. Testosterone-treated and C males had similar antibody concentrations on D39. Concentrations decreased in both groups between D39 and D55 (upper panel, Fig. 3; date effect: $F_{1,18}$=49.3, P<0.0001) and the magnitude of this decrease was larger in T than C males (date × treatment interaction: $F_{1,18}$=7.2, P=0.015). As a result, T males had lower antibody concentrations than C males on D55.

Cell-mediated immunity

Wing web thickness of C and T males prior to the D63 challenge PHA injection did not differ (C males: 0.598±0.049 mm; T males: 0.613±0.033 mm; medians ±0.5 interquartile intervals). Swelling measured on D64, D65 and D66 changed from one day to another (lower panel, Fig. 3; $F_{2,36}$=11.1, P<0.0002) in a hormone treatment-related manner (time × treatment interaction: $F_{2,36}$=4.0, P<0.03). Swelling in C males did not differ between D64 and D65, but decreased between D65 and D66. By contrast, swelling in T males decreased between D64 and D65 and then did not change between D65 and D66. This different time course resulted in less swelling in T than C males on D65.

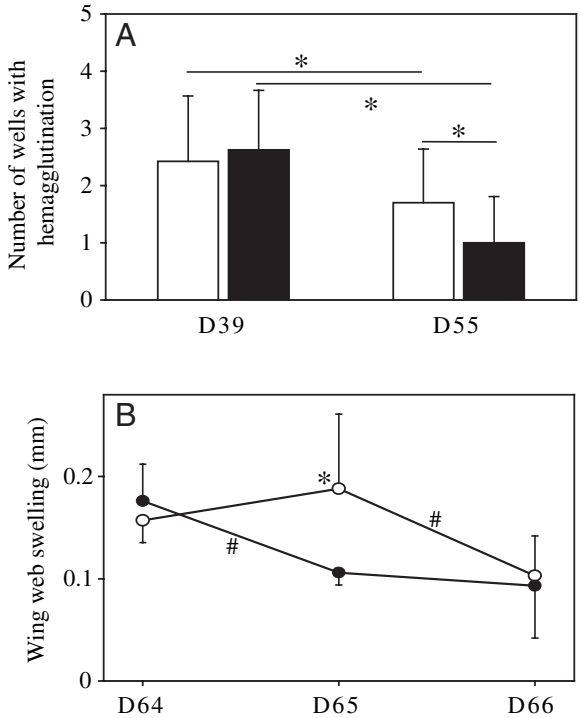

Fig. 3. (A) Humoral immunity (plasma heterologous antibody concentration measured as the number of plate wells with hemagglutination; means + S.D.) in control (white columns; N=10) and testosterone- (T) treated (black columns; N=10) adult male house finches five (D39) and 21 (D55) days after the fourth injection of antigen (washed sheep red blood cells). Samples were collected 39 (D39) and 55 (D55) days after initiation of the T or control treatment. *Indicates a significant (P<0.05) difference between two groups (Student–Newman–Keuls test). (B) Cell-mediated immunity (difference in wing web thickness from day 63; medians ±0.5 interquartile intervals) measured one (D64), two (D65) and three (D66) days after one local injection of phytohemaglutinin to testosterone-treated (filled circles) and control (open circles) males. *Indicates a statistically significant difference between C and T males. #Indicates a difference between time points within a same group. See legend of A for additional comments.

Lymphocyte numbers

Circulating lymphocyte concentrations changed between D-3 and D55 ($F_{4,72}$=4.1, P<0.005; Fig. 4), being higher 1 week after the first SRBC injection (D20) than at other times (Student–Newman–Keuls tests: P<0.05). Lymphocytes numbers did not change between D-3, D10, D39 and D55 and were not influenced by T administration.

Discussion

The role of T in avian immunity remains a matter of debate because some investigations found immunosuppressive effects of T while other studies found no such effects (Introduction) and neither the basis for this difference nor the mechanisms involved are well understood. Previous work suggested that an understanding of the effects of hormones on

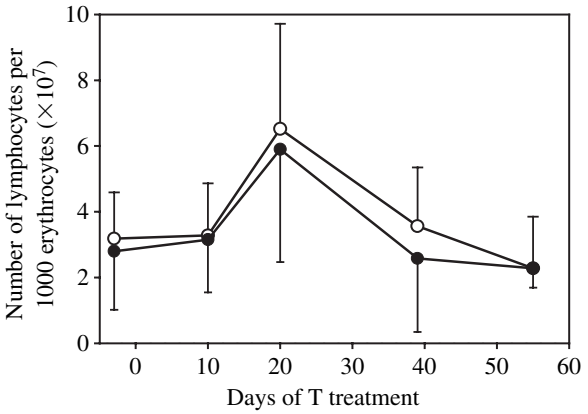

Fig. 4. Number of lymphocytes (medians ±0.5 interquartile intervals) per 1000 erythrocytes counted on stained thin blood smears of adult male house finches treated with testosterone-filled (black circles; *N*=10) or control (white circles; *N*=10) Silastic capsules. Capsules were implanted on Day 0. All birds received one weekly injection of sheep red blood cells for 4 weeks starting on Day 13.

immunocompetence requires the determination of multiple indices of immunity (Lochmiller, 1995; Sheldon and Verhulst, 1996; Zuk and Johnsen, 1998). To this effect, we characterized the influence of T administration on immune responses in adult male house finches using three different parameters: humoral and cell-mediated immunity and lymphocyte numbers. This administration influenced two parameters (humoral and cell-mediated immunity) in a manner consistent with the hypothesis that T is immunosuppressive.

Three independent measures confirmed the effectiveness of the T treatment. First, T-filled Silastic capsules weighed less at the end than at the beginning of the study, indicating T release. Second, T-treated males had higher plasma T levels than C males for the duration of the experiment. These levels (25–30 ng ml^{-1}) in hormone-treated finches were higher than those reported in free-ranging conspecific males (Duckworth et al., 2004; Stoehr and Hill, 2000, 2001). However, we found no evidence suggesting that the T treatment induced pathological effects such as a decrease in body mass or mortality. Furthermore, differences in T levels between studies should be interpreted with caution as these levels were often measured in different laboratories and using different types of assays (ELISA *vs* radioimmunoassays). Finally, finches receiving T-filled capsules had larger CPs, an androgen-dependent secondary sexual characteristic, than control finches. This effect was specific as shown by the fact that T treatment had no effect on body condition as estimated by body mass.

The methodology of the present investigation differed in several respects from that of previous avian studies examining the production of heterologous antibodies to assess the endocrine regulation of humoral immunocompetence (Donker and Beuving, 1989; Selvaraj and Pitchappan, 1985) and was designed to maximize immune responses of the experimental subjects. In particular, house finches used here received an initial injection of SRBC in Complete Freund's adjuvant followed with three additional injections of SRBC. Multiple injections may have induced stronger and different antibody responses, as well as a higher proportion of birds producing antibodies, than a single (Casto et al., 2001; Donker and Beuving, 1989; Duffy et al., 2000; Peters, 2000) or two (Evans et al., 2000; Von Schantz et al., 1999) consecutive injections of antigen, especially in cases where no adjuvant was used. Evidence in support of this hypothesis is provided by the fact that the blood of all experimental finches contained antibodies to SRBC 5 days after the fourth SRBC injection and antibodies were detected in 80% of the finches 2 weeks after this injection. At this time average antibody concentrations had decreased, but were correlated with those 2 weeks earlier. Thus, the apparent lack of antibody production by some individuals in previous studies may have been related to the immunization protocol rather than from inherent lack of response to the antigen. It is of interest that no anti-SRBC antibodies were detected in the blood of C or T finches 1 week after the first SRBC injection. This finding contrasts with that of Peters (2000) who reported maximal antibody titers in superb fairy-wrens 9 days post-immunization. The difference between studies may have resulted either from species differences in immunocompetence, from differences in conditions experienced by the birds (see below), or from the fact that immune responses to SRBC develop slower when these cells are administered in adjuvant (present investigation) than in phosphate-buffered saline solution (Peters, 2000).

Testosterone-treated males had evenly high circulating concentrations of the steroid for the duration of the study, yet the influence of this treatment on humoral and cell-mediated immunity was time-dependent. In particular, plasma anti-SRBC antibody concentrations were lower in T than C males on D55. Furthermore, local inflammatory response to PHA injection in C males persisted for 2 days post-injection and then subsided. By contrast, inflammation in T males decreased between 1 and 2 days after the injection. Thus, C and T males apparently mounted similar immune responses to SRBC and to PHA administration, but T administration compromised the maintenance of these responses.

Several mechanisms may account for the observed time-dependent effects of T on house finch immunity. As far as humoral immunity is concerned, T may have impaired the ability of birds to produce additional antibodies at the conclusion of the immunization period. Testosterone administration may also have accelerated the degradation or elimination of antibodies produced during and shortly after this period. Finally, the hemagglutination test used in the present study is not specific for antibody (immunoglobulins, Ig) types and detects IgG as well as IgM proteins. Testosterone may differently affect the production and degradation rates of these antibodies. Regardless of the mechanisms involved, the data emphasize the importance of measuring the dynamics of T effects on various immune responses rather than determining these responses at a single time point.

Previous studies investigated the mechanisms that mediate the immunosuppressive influence of T (e.g. Owen-Ashley et al., 2004), but these mechanisms remain poorly understood. One hypothesis is that T directly affects cells of the immune system. Supporting this hypothesis, lymphocytes in fish (Slater et al., 1995) and the bursa of Fabricius in immature chickens (Sullivan and Wira, 1979) have androgen receptors. *In vitro* studies using mammalian cells found T to suppress the production of antibodies by blood mononuclear cells (Kanda et al., 1997), the proliferation of lymphocytes induced by mitogens (Ahmed et al., 1987; Kotani et al., 1974; Lehmann et al., 1988) and the survival of leukocytes (Slater and Schreck, 1997). Testosterone also reduced the *in vitro* production of prostaglandin E2, an important mediator of local inflammatory responses, by human monocytes (Miyagi et al., 1993). Evidence for similar effects of T in birds is, however, currently lacking and little experimental supports the idea in these organisms that T directly influences cells of the immune system.

Alternatively, effects of T on immune function in birds may be indirect and mediated by the main adrenal glucocorticoid, CORT. Corticosterone in birds is generally immunosuppressive (Marsh and Scanes, 1994). This steroid is immunosuppressive also in some (rodents: Collins and Deas, 1986; Stewart et al., 1988; review: Sapolsky, 1992) although not other vertebrates, in which it rather enhances immunocompentence (side-blotched lizard, *Uta stansburiana*: Svensson et al., 2002). Although CORT administration to chickens did not decrease their production of heterologous antibodies in response to a single antigen injection (Donker and Beuving, 1989), this treatment to mallards, *Anas platyrhynchos*, depressed innate as well as humoral immunity (Fowles et al., 1993). Plasma T and CORT levels in intact male passerines are seasonally correlated (Deviche et al., 2000; Klukowski et al., 1997; Johnsen, 1998) and T treatment increased plasma CORT levels and/or the concentration of plasma binding protein for this steroid in several avian species (house sparrow: Evans et al., 2000; dark-eyed junco: Schoech et al., 1999; Deviche et al., 2001b; European starling: Duffy et al., 2000; song sparrow: Owen-Ashley et al., 2004) including the house finch (Deviche et al., 2004). Taken together, these findings are consistent with the hypothesis that immunosuppressive effects of T in adult birds result from androgen-mediated elevated CORT secretion as recently proposed by Evans et al. (2000). This mechanism may involve a modulation by T of CORT actions on immune cells because *in vivo* androgen treatment to immature chicks reduced their bursa of Fabricius cell concentration of cytosolic glucocorticoid receptors (Coulson et al., 1982).

As already indicated (Introduction), studies examining effects of T on avian immunity have provided equivocal results. In addition to procedural differences, such as those described above, some of the observed variation may relate to the fact that immunocompetence can be modulated by various environmental and social factors (e.g. lizards: effect of cold: Svensson et al., 1998; effect of social factors: Svensson et al., 2001) that can in some cases themselves influence the activity of the reproductive system (Leitner et al., 2003; Wingfield et al., 2003). In addition, immune responses have a genetic, heritable component (blue tit, *Parus coeruleus*: Råberg et al., 2003; lizards: Svensson et al., 2001). Thus, effects of T on immune functions, whether direct or indirect (see above), may depend on complex interactions between endocrine and non-endocrine factors including environmental and genotypic variables.

The present investigation found no influence of T treatment on lymphocyte counts. Studies on this subject have yielded equivocal results. Testosterone administration to red jungle fowl, *Gallus gallus*, decreased lymphocyte counts (Zuk et al., 1995), but increased these counts without affecting the numbers of other leukocyte types in adult house sparrows (Nava et al., 2001). In intact barn swallows, *Hirundo rustica*, T levels and lymphocyte counts were unrelated (Saino and Møller, 1995). Several factors including age and reproductive status may account for differences between studies. For example, T administration increased lymphocyte numbers in adult but not juvenile sparrows (Nava et al., 2001). Also, lymphocyte counts and the size of the comb, a T-dependent (Ligon et al., 1990) and sexually selected trait, were positively correlated in red jungle fowl before the breeding season, but negatively correlated during this season (Zuk and Johnsen, 1998). All finches used in the present study were adults that we exposed to short days to prevent reproductive development. Regardless of whether finches had low or high circulating T levels, lymphocyte counts were higher 1 week after the first SRBC injection than at other times. We speculate that this increase was in response to the solvent (Freund's Complete Adjuvant) itself or to the presence of a foreign antigen (SRBC). The finding that T treatment did not alter lymphocyte counts must be interpreted conservatively because the sampling technique resulted in our counting relatively small numbers of lymphocytes for each bird and counts within a group on a given date were quite variable (see error bars on Fig. 4). Small lymphocyte counts and large inter-individual variation in these counts may have hampered the detection of subtle effects of T administration on this parameter.

To conclude, we found experimentally elevated circulating T levels in male house finches to suppress humoral immunity in response to a heterologous antigen and cell-mediated immunity in response to a foreign protein. These results confirm and extend previous findings and indicate that T can be immunosuppressive, a conclusion that supports the ICH hypothesis. The data add to the increasing evidence in birds that T exerts a great diversity of behavioral and physiological effects. Circulating T levels in most seasonally breeding male birds naturally undergo dramatic natural seasonal changes. In the future, these birds will likely continue to serve as choice models for investigating the environmental regulation of reproductive endocrinology, the role of gonadal hormones in the control of the immune functions and the mechanisms that mediate this role.

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