Effects of photoperiod history on immune responses to intermediate day lengths in Siberian hamsters (Phodopus sungorus)

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Abstract

Seasonal changes in day length enhance or suppress immune function in individuals of several mammalian species. Siberian hamsters (Phodopus sungorus) are long-day breeders that adjust reproductive physiology and behavior, body mass, and immune function following exposure to short photoperiods. Photoperiods of intermediate-duration, encountered in nature by juvenile hamsters born in early-spring and by those born in mid-summer, trigger gonadal development in the former cohort and inhibit the onset of puberty in the latter. Divergent reproductive responses to the same intermediate photoperiod depend on a photoperiod history, communicated during gestation. These experiments assessed whether photoperiod history during gestation likewise impacts immunological responses to intermediate photoperiods. Male hamsters were gestated in long photoperiods and remained in long photoperiods postnatally, or were transferred to an intermediate-duration or a short-duration photoperiod; other males were gestated in short days and transferred to an intermediate-duration photoperiod at birth. Long days stimulated, and short days inhibited, somatic and reproductive development; intermediate day lengths either accelerated or inhibited somatic and reproductive development, depending on whether hamsters were gestated in short days or long days, respectively. By contrast, photoperiod during gestation did not affect most immune endpoints. The data suggest that photoperiodic mechanisms that enhance and suppress several aspects of immunity in young-adult hamsters are not responsive to prenatally communicated photoperiod history information.

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1. Introduction

Environmental factors such as food availability, humidity, and ambient temperature impose constraints on survival and reproduction and often vary on a seasonal basis (Goldman, 2001; Nelson et al., 2002). Mechanisms have evolved among many mammalian species that permit individuals to anticipate changes in environmental conditions weeks-to-months in advance and thereby engage physiological mechanisms that promote survival and successful reproduction (Prendergast et al., 2002). Changes in reproductive physiology that occur in many non-tropical vertebrates are among the most-studied seasonal adaptations (Bronson, 1989). Among temperate and boreal-zone mammals, for example, the majority of offspring produced in a given year are born during the spring when food is abundant and temperatures are relatively moderate (Bronson, 1989). The seasonal change in day length (photoperiod) provides an indication of time-of-year; photoperiodic entrainment of an endogenous circadian rhythm in pineal melatonin secretion evokes changes in many seasonal adaptations, including reproduction and metabolism (Bronson, 1989; Goldman, 2001; Prendergast et al., 2002). Seasonal changes also occur in the immune systems of several vertebrate species, presumably to facilitate survival in the face of annual cycles in pathogen prevalence and/or energy availability (Ross et al., 1993; Nelson et al., 2002; Dowell et al., 2003; Møller et al., 2003). Several recent studies, principally in seasonally breeding rodents, have described robust effects of experimental changes in day length on in vitro and in vivo measures of immunity (Demas and Nelson, 1996; Yellon et al., 1999; Prendergast et al., 2002; Bilbo et al.,
studies of immunological photoperiodism in this species to effects of endotoxemia (Prendergast et al., 2003). Because all 32 (Gorman and Zucker, 1998; Gorman and Lee, 2002). during late summer initiate somatic and gonadal regression, transitions. Rather, the intermediate photoperiods occurring relevant environmental triggers for photoperiodic seasonal tory experiments on photoperiodism—are unlikely to be the hood that very short photoperiods—typical of many labora- of photoperiod-history effects is underscored by the likeli- (e.g., 16L) photoperiod, an intermediate (e.g., 14L) photope- iod is interpreted as relatively shorter, and reproductive and somatic inhibition ensues (Prendergast et al., 2000; Kauffman and Zucker, 2002). Dams communicate an analogous photo- period history to fetuses in utero (Weaver et al., 1987; Stetson et al., 1989). Photoperiod exposure during gestation deter- mines somatic and reproductive responses to intermediate day lengths during puberty; i.e., juveniles gestated in 8L exhibit somatic and gonadal growth, whereas those gestated in 16L defer reproductive development, when reared in 14L (Weaver and Reppert, 1986). History-dependent reproductive responses to intermediate-duration photoperiods ensure re- productive stimulation in response to photoperiods occurring in late spring and reproductive inhibition in response to identical photoperiods occurring in late summer, and thus permit seasonally appropriate responses to ambiguous photo- periods (Prendergast et al., 2000). The ecological importance of photoperiod-history effects is underscored by the likeli- hood that very short photoperiods—typical of many labora- tory experiments on photoperiodism—are unlikely to be the relevant environmental triggers for photoperiodic seasonal transitions. Rather, the intermediate photoperiods occurring during late summer initiate somatic and gonadal regression, such that the “short-day” phenotype is achieved weeks in advance of the actual appearance of very short DLs (e.g., 8L) (Gorman and Zucker, 1998; Gorman and Lee, 2002).

Siberian hamsters exhibit a number of immune system changes in response to changes in photoperiod. Multiple adjustments in immune function follow the transition from long to short photoperiods, including decrements in T-cell-dependent antibody production (Yellon et al., 1999; Drazen et al., 2000), increases in the number of T cells and in several subtypes of circulating leukocytes (Bilbo et al., 2002), decreases in the magnitude and duration of sickness behav- iors (Bilbo et al., 2002), and protection against the lethal effects of endotoxemia (Prendergast et al., 2003). Because all studies of immunological photoperiodism in this species to date have used either very long (≥ 15L) or very short (≤ 10L) photoperiods to induce changes in immunity, it is not known (1) whether intermediate photoperiods (e.g., 14L) are sufficient to trigger photoperiodic changes in immunity, and (2) whether immunological responses to intermediate photoperiods occur in a history-dependent manner, similar to that which governs somatic and reproductive responses. To address these issues, the following experiments used a photoperiod-history paradigm in which dams communicate gestational photoperiod information to their fetuses—information that alters somatic and reproductive responses to intermediate photoperiods during juvenile development (Weaver et al., 1987; Stetson et al., 1989)—to test the hypothesis that photoperiod history affects immune res-ponses to intermediate day lengths.

2. Material and methods

2.1. Animals and photoperiod manipulations

Male Siberian hamsters (Phodopus sungorus) from a breeding colony maintained at The Ohio State University were used in all experiments. Breeding pairs were main- tained under illumination conditions that provided either a long-duration (16 h light/day; LD) photoperiod or a short- duration (8 h light/day; SD) photoperiod. Pairs were inspected daily for the presence of pups, and the day of birth was designated as day 0. Photoperiod transfers were performed on day 0 as follows: LD dams and their pups were transferred from the LD photoperiod into rooms that provid- ed either an LD photoperiod (LD–LD), a SD photoperiod (LD–SD), or an intermediate-duration (IntD; 14 h light/day) photoperiod (LD–IntD); SD dams and their pups were transferred from the SD photoperiod into a room that provided an IntD photoperiod (SD–IntD). The time of the onset of darkness was the same for all pre- and post-natal photoperiod treatments (1500 h E.S.T.). Hamster pups were weaned at 18 days of age (day 18) and were kept in their respective postnatal photoperiods for the remainder of the experiment. Male offspring were housed in sibling pairs or triads in polypropylene cages (27.8 × 7.5 × 13 cm) under conditions of constant temperature and humidity of 21 ± 4 °C and 50 ± 10%, respectively, with ad libitum access to food (Harlan Teklad 8640 rodent diet, Indianapolis, IN) and filtered tap water. Separate groups of hamsters were used in Experiments 1 and 2. All procedures in these experiments were conducted in accordance with the National Institutes of Health guidelines for the use of experimental animals, and the protocols were approved by the local Institutional Ani- mal Care and Use Committee.

2.2. Experiment 1. Photoperiod history effects on circulating leukocytes and DTH responses

2.2.1. Experimental protocol

At 35 days of age, blood samples for leukocyte analyses were collected from male hamsters in each of the four
samples (esters were anesthetized with isoflurane vapors, and blood samples (~ 250 μl) were obtained via the retro-orbital sinus using 270-μl Natleson collection tubes. Samples were transferred to sterile microcentrifuge tubes containing 30 μl heparin, mixed thoroughly, and held at room temperature until leukocyte analyses were performed (see Section 2.2.3 ). Following blood collection, hamsters were administered 0.5 ml sterile 0.9% saline s.c. Blood collection was performed in a room separate from the general animal colonies. Handling was kept consistent and time to a minimum (<1 min), and following blood collection, hamsters were not returned to their respective photoperiod chambers until all blood collection was completed for a given day. Beginning at 40 ± 2 days of age (hereafter, day 40), hamsters were sensitized to DNFB on two consecutive days with 0.5% DNFB (in oil/acetone; see Section 2.2.4; Bilbo et al., 2002); DTH reactions were elicited on day 46.

2.2.2. Somatic and reproductive measures

Body masses (± 0.1 g) and estimated testes volumes (ETV; calculated as L × W^2 [in mm] of the left testis; Gorman and Zucker, 1995) were obtained in lightly anesthetized hamsters on day 40. ETV is positively correlated (R=0.9) with testis weight (Gorman and Zucker, 1995) and therefore provides a valid indicator of reproductive status.

2.2.3. Leukocyte analyses

Total leukocyte numbers and lymphocyte-neutrophil differentials were obtained from whole blood samples on a hematology analyzer (F800, Sysmex, McGraw Park, IL) according to methods previously reported for this species (Bilbo et al., 2002). Specific leukocyte subtypes were measured by immunofluorescent antibody (Ab) staining and analysis by using single color flow cytometry (FACS-Calibur, Becton Dickinson). Lymphocyte, neutrophil, and monocyte subpopulations were identified and gated by using forward- versus side-scatter characteristics. T cells were identified by using CyChrlabeled anti-CD3 (clone 145-2C11), B cells by using phycoerythrin-labeled anti-B220 (RA3-6B2), and natural killer (NK) cells by using phycoerythrin-labeled anti-NK1.1 (PK136). Neutrophils and monocytes were identified by using forward- versus side-scatter patterns and allophycocyanin-labeled anti-CD11b (M1/70). L-selectin and CD44-positive cells were identified by using phycoerythrin-labeled anti-CD62L (MEL-14) and FITC-labeled anti-CD44 (IM7), respectively. Each staining panel consisted of a single Ab. All monoclonals were directly conjugated, rat anti-mouse Abs (except for NK1.1, a murine Ab) and were obtained from Becton Dickinson-PharMingen. In brief, blood samples were incubated with Ab for 20 min at room temperature, washed with PBS, and read on the FACSCalibur; 3000–5000 events were acquired from each preparation. Control samples matched for each fluorochrome and each Ab isotype were used to set negative staining criteria. Because Abs directed against hamster immune cells were not available, we adapted crossreactive anti-mouse Abs for these analyses. Ab clones from different suppliers were screened. Staining patterns and relative percentages observed with crossreactive clones were similar to those observed in mice whereas non-crossreactive clones showed no positive staining. Relative percentages of independently run T-, B-, and NK-cell panels accounted for >95% of positively stained cells in the lymphocyte gate. Data were analyzed by using Cellquest software (Becton Dickinson).

2.2.4. Induction of DTH

DTH was induced by application of the antigen, 2,4-dinitro-1-fluorobenzene (DNFB; Sigma), to the pinnae of each hamster after initial immunization (“sensitization”) by application of DNFB to the dorsum (Bilbo et al., 2002). Sensitization was induced and DTH elicited as follows: on day 40, all hamsters (DNFB naive) were anesthetized with isofluorane vapor, and an area of 2 × 3 cm was shaved on the dorsum. Twenty-five microliters of DNFB [0.5% (w/v) in 4:1, acetone/olive oil vehicle] was applied to the dorsal skin on two consecutive days. On day 46, baseline thickness of both pinnae was measured in lightly anesthetized hamsters prior to induction of DTH by using a constant-loading dial micrometer (Mitutoyo, Tokyo). Immediately after baseline pinna measurements were obtained, 20 μl of DNFB [0.2% (w/v) in 4:1, acetone/olive oil] was applied to the skin of the dorsal surface of the right pinna. Left pinnae were treated with vehicle. Pinna thickness was measured every 24 h for the next 5 days. Pinna thickness values obtained on each day following challenge were expressed as a percentage of baseline thickness for statistical calculations. All DNFB treatments and pinna measurements were performed between 1400 and 1500 h, and all measurements were made on the same relative region of the pinna.

2.3. Experiment 2. Photoperiod history effects on antigen-specific antibody production

2.3.1. Experimental protocol

At 25 days of age, male hamsters in each of the four experimental groups (LD–LD, n = 16; LD–SD, n = 16; LD–IntD, n = 20; SD–IntD, n = 14) were lightly anesthetized with isofluorane vapors and inoculated with KHL (100 μg KHL protein in 0.1 ml sterile 0.9% saline, s.c.) in order to elicit a primary humoral antibody response (see Section 2.3.3). KHL is a respiratory protein of the giant keyhole limpet (Megathura crenulata) and was used because it generates a robust antigenic response in rodents, but does not cause inflammation, prolonged fever, or illness (Dixon et al., 1966; Drazen et al., 2000). Blood samples (270 μl) were obtained under light isofluorane anesthesia via the right retroorbital sinus using heparinized collection tubes on days 32, 36, 39, and 46; these intervals were chosen to capture a
profile of immunoglobulin production during the course of the immune response. Blood samples were allowed to clot at room temperature for 1 h, and then were centrifuged at 4 °C for 30 min at 2500 r.p.m., and the supernatant was frozen at −70 °C until assayed for anti-KLH IgG and IgM via ELISA (see below).

2.3.2. Somatic and reproductive measures

Reproductive (ETV) and somatic (body mass) measures were obtained under isoflurane anesthesia on days 25, 32, 36, 39, and 46.

2.3.3. KLH ELISA

Serum concentrations of anti-KLH IgG and IgM were determined using an ELISA as described in detail elsewhere (Demas et al., 1997). Thawed serum samples were diluted with PBS-Tween, and 150 μl of each serum dilution was added in duplicate to the wells of KLH-coated microtiter plates. Positive control (pooled serum from hamsters previously determined to have high levels of anti-KLH antibodies) and negative control (pooled serum from hamsters injected with sterile saline vehicle) samples were also added in duplicate to each plate. The plates were sealed, incubated, and washed before addition of 2° Ab (alkaline phosphatase-conjugated anti-mouse IgG or IgM). Plates were again incubated and washed, and then treated with the enzyme substrate (p-nitrophenyl phosphate). After 20 min, the enzyme reaction was stopped and the optical density (OD) of each well was determined using a plate reader equipped with a 405-nm wavelength filter. Average OD for duplicate wells was expressed as a percentage of its plate-positive control OD value for statistical analyses (Drazen et al., 2000).

2.3.4. Data analyses

In Experiment 1, leukocyte concentrations, body masses, and ETVs were compared using ANOVA and, where justified by a significant F-statistic, between-groups comparisons were made using a Bonferroni–Dunn test. DTH reactions (changes in pinna thickness) were compared between-groups using repeated-measures ANOVA (Statview 5; SAS Institute, Cary, NC, USA) with photoperiod treatment (LD–LD, LD–SD, LD–IntD, SD–IntD) as a between-subjects factor. Post hoc comparisons on daily mean pinna thicknesses were performed using Tukey–Kramer HSD tests (Bilbo et al., 2002). In Experiment 2, anti-KLH antibody profiles were compared using repeated-measures ANOVAs with photoperiod treatment as a between-subjects factor. Post hoc between-subjects comparisons on daily antibody values were performed using two-tailed t-tests. Alpha was set at 0.05. Given the large number of post hoc comparisons, a Bonferroni–Dunn correction ([(alpha]/[number of pairwise comparisons]) was applied to all statistical tests; thus comparisons were not considered significant unless the corresponding p-value was less than 0.0083.

3. Results

3.1. Experiment 1

3.1.1. Somatic and reproductive measures

Hamsters gestated in LD and housed in LD postnatally (LD–LD) had significantly higher ETV relative to LD-gestated hamsters housed postnatally in SD (LD–SD) (p<0.0001; Fig. 1A). The testes of LD-gestated hamsters housed in IntD postnatally (LD–IntD) were smaller than those of LD–LD hamsters (p<0.0001), but were significantly larger than those of LD–SD hamsters (p=0.0001). In contrast, the testes of SD-gestated hamsters reared in IntD (SD–IntD) were significantly larger than those of both LD–IntD (p<0.0001) and LD–LD hamsters (p<0.0083). Group differences in body weights followed a pattern similar to that of ETV, except body weights of SD–IntD hamsters were statistically comparable to those of LD–LD hamsters (Fig. 1B).

3.1.2. Leukocyte numbers

Total white blood cell counts were higher in LD–SD hamsters relative to LD–LD hamsters (p<0.0001; Fig. 2A). LD–IntD hamsters exhibited white blood cell counts that were significantly lower than those of LD–SD hamsters (p<0.0001), but did not differ from those of LD–LD hamsters (p<0.0083). White blood cell counts of SD–IntD were likewise comparable to those of LD–LD hamsters and did not differ from those of LD–IntD hamsters (p>0.4). Total lymphocyte numbers were significantly higher in LD–SD hamsters relative to LD–LD hamsters (p<0.0001; Fig. 2B), and LD–IntD and SD–IntD groups exhibited comparable total lymphocyte counts (p>0.1). Total numbers of monocytes were significantly higher in LD–SD hamsters relative to LD–LD hamsters (p<0.0001; Fig. 2C). LD–

![Fig. 1. Mean (+ SEM) estimated testis volumes (A) and body mass (B) on day 40 of male Siberian hamsters in Experiment 1. Hamsters were gestated in LD (16 h light/day) and either remained in the LD photoperiod postnatally (LD–LD), or were transferred on the day of birth (day 0) to either a SD (8 h light/day) photoperiod (LD–SD) or an IntD (14 h light/day) photoperiod (LD–IntD); SD–IntD hamsters were gestated in SD and transferred to IntD on the day of birth. *p<0.0083 vs. all other groups.](image-url)
IntD hamsters exhibited circulating monocyte numbers that were significantly lower than those of LD–SD hamsters ($p < 0.0001$), but higher than those of LD–LD hamsters ($p < 0.0001$). Monocyte counts of SD–IntD were comparable to those of LD–IntD hamsters ($p > 0.0083$) but did not differ significantly as a result of any other photoperiod manipulations ($p > 0.0083$, all comparisons; Fig. 2D). Comparisons of photoperiod effects on additional specific lymphocyte subsets (T cell, B cell, NK cell) and CD44 and CD62L-positive cells are presented in Table 1.

### 3.1.3. DTH

LD–SD hamsters exhibited a larger DTH response relative to all other groups, which did not differ from each other in the overall pattern of the inflammatory response (Fig. 3).

#### 3.2. Experiment 2

##### 3.2.1. Somatic and reproductive responses

The pattern of testicular development differed significantly across photoperiod treatment groups ($F_{3,63} = 125.1$; $p < 0.0001$). Between day 25 and day 46, LD–SD hamsters had significantly smaller testes relative to all other groups. In LD-gestated hamsters, exposure to IntD postnatally inhibited gonadal development relative to that observed in LD–LD hamsters ($p < 0.0083$; Fig. 4A). Between day 25 and day 46, LD–SD hamsters had significantly smaller testes relative to all other groups. In LD-gestated hamsters, exposure to IntD postnatally inhibited gonadal development relative to that observed in LD–LD hamsters ($p < 0.0083$; Fig. 4A).
served in LD-reared hamsters (p < 0.0001). In contrast, postnatal exposure of SD-gestated hamsters to IntD was compatible with full gonadal development that did not differ from that observed in LD-reared hamsters (p > 0.6).

The patterns of body weight accretion differed significantly between LD–LD and LD–SD groups, and between LD–IntD and SD–IntD groups (p < 0.0001, both comparisons; Fig. 4B).

3.2.2. Antibody production

Peak anti-KLH IgM antibody production occurred 7 days following inoculation (day 32). Pre- and postnatal photoperiod had no significant effect on IgM antibody production (p > 0.05, all comparisons; Fig. 5A). Peak anti-KLH IgG antibody production occurred 21 days following inoculation (Fig. 5B). IgG production was enhanced in LD–IntD hamsters relative to LD–SD hamsters (p < 0.0001); none of the remaining pairwise comparisons indicated significant effects of photoperiod treatments on the overall pattern of IgG production (p > 0.0083, all comparisons). On the day of peak IgG antibody production (day 46), IgG values of LD–LD hamsters were significantly higher than those of LD–SD hamsters (p < 0.0083); LD–IntD IgG concentrations also significantly exceeded those of LD–SD on day 46, but were comparable to those of SD–IntD hamsters.

3.2.2. Antibody production

Peak anti-KLH IgM antibody production occurred ≤ 7 days following inoculation (day 32). Pre- and postnatal photoperiod had no significant effect on IgM antibody production (p > 0.05, all comparisons; Fig. 5A). Peak anti-KLH IgG antibody production occurred 21 days following inoculation (Fig. 5B). IgG production was enhanced in LD–IntD hamsters relative to LD–SD hamsters (p < 0.0001); none of the remaining pairwise comparisons indicated significant effects of photoperiod treatments on the overall pattern of IgG production (p > 0.0083, all comparisons). On the day of peak IgG antibody production (day 46), IgG values of LD–LD hamsters were significantly higher than those of LD–SD hamsters (p < 0.0083); LD–IntD IgG concentrations also significantly exceeded those of LD–SD on day 46, but were comparable to those of SD–IntD hamsters.

Fig. 4. Mean (± SEM) estimated testis volumes (A) and body mass (B) on of male Siberian hamsters in Experiment 2. Group abbreviations as in Fig. 1, *p < 0.0083 vs. all other groups; §p < 0.0083 vs. both LD–LD and SD–IntD (between-groups analyses).

Fig. 5. Mean (± SEM) anti-KLH (A) IgM and (B) IgG antibody concentrations (expressed as a percentage of plate positive controls) in serum obtained 7, 11, 14, and 21 days following inoculation on day 25 with KLH in Experiment 2. Group abbreviations as in Fig. 1. *p < 0.0083 vs. LD–SD.
4. Discussion

Consistent with previous reports in this species (Weaver et al., 1987; Stetson et al., 1989), a prenatally encoded photoperiod history altered somatic and reproductive responses to an intermediate-duration photoperiod. Postnatal exposure to SD inhibited reproductive and somatic development relative to postnatal LD exposure. However, when raised from birth in identical intermediate photoperiods, male Siberian hamsters that were gestated in LD exhibited smaller reproductive organ sizes and body masses relative to hamsters gestated in SD. Indeed, testis sizes and body masses of SD–IntD hamsters were indistinguishable from those of LD–LD hamsters, and reproductive and somatic measures of LD–IntD hamsters were significantly lower than those of LD–LD hamsters. In none of the immune endpoints measured (13 enumerative leukocyte measures, two functional assays of immune responses) did gestational photoperiod reverse the postnatal response to the intermediate-duration photoperiod in a direction similar to that observed for reproductive and somatic measures. Although six measures of leukocyte numbers revealed a difference between LD–LD and LD–SD hamsters (total leukocytes, total lymphocytes, total monocytes, CD62L monocytes, CD44 monocytes, and CD62L neutrophils), on each of these measures, the respective values were indistinguishable between LD–IntD and LD–SD hamsters. DTH reactions were significantly enhanced in LD–SD hamsters relative to LD–LD hamsters, yet LD–IntD and SD–IntD hamsters exhibited comparable inflammatory responses, similar in magnitude to those of LD–LD hamsters. Although IgM values did not differ across photoperiod treatments, peak antigen-specific IgG antibody production was higher in LD–LD hamsters relative to LD–SD hamsters (cf. Drazen et al., 2000), but did not differ in IntD hamsters as a function of gestational photoperiod. Together, the data indicate that a number of immune measures are responsive to changes in photoperiod in young Siberian hamsters in a manner similar to that observed in adults of this species (Yellon et al., 1999; Bilbo et al., 2002); however, unlike photoperiodic systems that govern reproductive and somatic development (Stetson et al., 1989), photoperiodic mechanisms that enhance and suppress immunity are not responsive to prenatally communicated photoperiod history information.

The immune responses to postnatal IntD exposure were comparable between groups of hamsters gestated in LD and those gestated in SD, but differed in their resemblance to SD-like and LD-like values on a trait-by-trait basis. For example, DTH responses were statistically indistinguishable between LD–IntD and SD–IntD groups, and were low (LD-like) in magnitude. Total leukocytes, lymphocytes, and CD62L+ neutrophils exhibited LD-like values in IntD-reared hamsters, whereas CD62L+ monocytes, comparable between LD–IntD and SD–IntD hamsters, were SD-like in number. Moreover, total monocyte numbers and CD44+ monocytes, also comparable among IntD-reared groups, exhibited an altogether unique value, intermediate between that of LD–LD and LD–SD hamsters. In addition to illustrating an independence from photoperiod history effects, these observations suggest that the critical day length (i.e., the photoperiod duration above or below which a photoperiodic immune response is elicited; Elliott, 1976; Nelson, 1999) differs between subsets of leukocytes, as previously reported for distinct endocrine measures in this species (e.g., follicle-stimulating hormone and prolactin; Duncan et al., 1985). Total monocytes and CD44+ monocytes, in contrast, may respond to changes in day length in a graded manner, i.e., achieving circulating numbers in inverse proportion to the number of hours of light in the photocycle.

As in previous studies of hamster immune function, photoperiodic changes in total blood lymphocytes and leukocytes correlated well with organismal immune responses. In the current experiment, hamsters reared in SD exhibited robust DTH inflammatory responses, whereas hamsters exposed to LD postnatally did not; DTH responses under IntD were comparable to those of LD hamsters, and did not differ as a function of photoperiod history. DTH reactions are antigen-specific cell-mediated immune responses, dependent on T-cell function and immunological memory. Although the number of T cells were significantly higher in SD–IntD hamsters relative to the other three groups, the CD3+ cell-surface marker does not distinguish between memory and naïve T cells, and thus this increase in CD3+ cells in SD–IntD hamsters may not be of significance toward understanding the mediation of the observed difference in the organismal immune response to DNFB challenge. In contrast, CD44+ monocytes and CD62+ neutrophils were significantly higher in LD-SD hamsters relative to all other groups. Leukocytes expressing L-selectin molecules (CD62) interact with the vascular endothelium during inflammatory responses (Chen et al., 1997), and CD44 expression is critical to leukocyte migration during the challenge phase of DTH responses (Brooke et al., 1999). The SD enhancement of DTH in the present study corresponds well with the observed increases in circulating leukocytes expressing CD62 and CD44 molecules (cf. Bilbo et al., 2002). The absence of photoperiod history effects on these subpopulations of leukocytes and on the organismal DTH is consistent with previous reports in this species (Bilbo et al., 2002) and suggests that CD62+ neutrophils and CD44+ monocytes may be critical to mediating the effects of photoperiod on DTH in this species.

The absence of photoperiod-history effects on immune responses to intermediate-duration photoperiods in the present paradigm does not preclude photoperiod history from influencing responses to intermediate photoperiods at other stages of development. Photoperiod history communicated by the dam during late gestation influences reproductive responses to intermediate-duration photoperiods encountered during juvenile development (Weaver et al., 1987; Stetson et al., 1989; present data). An analogous photoperiod history can also be encoded during adulthood (Duncan and
Goldman, 1986; Prendergast et al., 2000; Kauffmann and Zucker, 2002), permitting inhibitory reproductive responsiveness to intermediate-duration photoperiods encountered after adult sexual development is complete. The present data do not exclude the possibility that photoperiod history encoded in adulthood impacts immune responses to intermediate day lengths. Even within the maternal-juvenile photoperiod history paradigm, evidence of photoperiod history effects can be transient and trait-specific. Raised in an intermediate photoperiod, male Siberian hamsters gestated in short, relative to long, days exhibit increases in circulating concentrations of follicle-stimulating hormone (FSH) and prolactin (PRL) at 22 days of age; this photoperiod history-dependent increase in FSH dissipates by 27 days of age, but increases in PRL persist through 52 days of age (Shaw and Goldman, 1995). Thus, the developmental time points at which immune parameters are assessed may ultimately dictate whether photoperiod history effects may be identified. This potential limitation may be more relevant to interpretation of the enumerative measures of immunity obtained via blood sampling on day 35, than to the measures of antibody production, which occurred over a longer time frame, or to the DTH responses, which integrate photoperiod information occurring over the course of many weeks (Prendergast et al., 2003).

The dissociation of immune responses from reproductive responses to intermediate photoperiods permits insights into the neuroendocrine mechanisms that may participate in seasonal changes in immunity in this species. Previous reports have suggested a causal (Bilbo and Nelson, 2001; Prendergast et al., 2002) or revealed a correlational (Bilbo et al., 2002) link between gonadal hormone secretion and photoperiodic changes in selected measures of immunity (in vitro lymphocyte blastogenesis, DTH responses) in Siberian hamsters. Despite pronounced differences in gonad size, however, male hamsters with different photoperiod histories exhibited comparable immune measures and responses when exposed to IntD in the present study, suggesting that photoperiodic changes in the activity of the hypothalamic-pituitary-gonadal axis are not sufficient to cause changes in these measures of immunity. Photoperiodic regulation of prolactin secretion also appears unlikely to participate in the phenomena described here. Photoperiod history encoded prenatally affects prolactin responses to intermediate day lengths for the first 2 months of life (Shaw and Goldman, 1995), an interval that overlapped with the developmental time points at which immune measures were obtained in the present study. Absent effects of photoperiod history on immune responses to intermediate photoperiods during this interval, the present data suggest that photoperiodic changes in prolactin secretion are likewise not sufficient to drive photoperiodic changes in any of several measures of immunity. Photoperiod-history-dependent changes in the duration of melatonin secretion (Shaw and Goldman, 1995) and/or in circulating concentrations of cortisol (Bilbo et al., 2002) remain plausible hormonal links between the neuroendocrine and immune systems for effecting photoperiodic changes in response to unambiguously long and short photoperiods.

From an information-processing perspective, the present data suggest that an individual’s history of photoperiod exposure plays little, if any, role in determining anticipatory seasonal changes in the immune system. In common with previous studies (Duncan et al., 1985; Hoffmann and Illnerova, 1986; Stetson et al., 1989), measures of body mass and reproductive physiology indicated that hamsters respond to intermediate photoperiods using antecedent photoperiod as a discriminating variable; however, such photoperiod history information either is not available or is not attended to by the immune system. The adaptive significance of photoperiodism lies in its conferring upon an individual the ability to anticipate seasonal changes in environmental variables that affect fitness (e.g., food, temperature; Prendergast et al., 2002); implicit in this link is the notion that anticipation is adaptive because changes in the photoperiodic trait do not occur instantaneously (e.g., deposition of fat, initiation of spermatogenesis). It may be that Siberian hamsters can enact large-scale changes (of the magnitude seen between LD and SD) in the immune system faster than they can enact changes in, for example, reproductive physiology. If seasonal changes in immunity could be engaged within a time interval that is substantially shorter than that required to engage, e.g., gonadal regression, then perhaps selective pressure to anticipate changes in photoperiod (or monitor direction of change in photoperiod) in a manner as precise and highly regulated as photoperiod-history-dependent regulation of the reproductive system is relaxed.

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