Form over Function?

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In this issue of the Journal of Biological Rhythms, Zhou et al. (2002) present data from several experiments aimed at identifying effects of photoperiod on the immune systems of two well-established photoperiodic model species: Siberian hamsters (Phodopus sungorus) and Syrian hamsters (Mesocricetus auratus). Their observations are unambiguous: after adaptation to short days, Syrian hamsters exhibited higher numbers of resident auricular lymphocytes, lower rates of spontaneous lymphocyte proliferation, and less interleukin-6 (IL-6) production. Short days had no apparent effect on a contact hypersensitivity (CH) response, on IgM or IgG primary antibody production, or on secondary proliferative responses to an antigen/irritant (dinitrofluorobenzene) in this species. Gonadectomy did not substantially alter the effect of short days on lymphocyte proliferation or on cytokine production. Siberian hamsters likewise exhibited relatively low basal rates of lymphocyte proliferation and IL-6 production in short days. Photoperiod also modified the response to a polyclonal T-cell mitogen in both sensitized and naive individuals, but had no effect on natural killer (NK) cell activity. The data clearly indicate that the immune systems of both Syrian and Siberian hamsters respond to photoperiod. However, based on these observations, the authors conclude that the expression of the immune response is not directly modified or compromised by photoperiod in either of these species. Instead, the authors contend that “photoperiod has no direct effect on immune responses per se in seasonal mammals” (p. 404). The report also reaches a wholly separate conclusion with regard to the role of the hormone leptin in mediating photoperiodic changes in immunity. Our analysis of the data discussed by Zhou et al. and recent work from our laboratory lead us to disagree with these conclusions.

Implicit in the authors’ discussion of the data, and in the conclusion that photoperiod has no direct effect on immune responses per se, are ambiguous distinctions between measures of the immune system (measures of relatively static traits considered to be components of the entire immune system [e.g., tissue weights, cell numbers], including traits of the adaptive and the innate immune system), immune responses (measures of adaptive changes in these traits in response to challenges [e.g., phagocytosis, fever dynamics, clonal selection of lymphocytes, lymphocyte trafficking]), and immune function (measures of the manner in which changes in adaptive and innate immune responses preserve host integrity). Zhou et al. (2002) provide evidence for effects of day length on immune measures (auricular lymphocyte counts) and responses (IL-6 production in sensitized lymphocytes, mitogen-stimulated T-cell blastogenesis). This is, of course, not the first report indicating effects of photoperiod on immune responses in a reproductively photoperiodic species (e.g., Demas and Nelson, 1996), nor is it the first report indicating the absence thereof (e.g., Yellon, Fagoaga, et al., 1999; Prendergast et al., 2001). We agree with the statement of Zhou et al. that it is important to discover...
any functional significance to the photoperiodic and melatonin-induced changes in immune measures and responses reported by many authors for several mammalian species (Blom et al., 1994; Demas et al., 1996; Demas and Nelson, 1998; Yellon, Fagoaga, et al., 1999; Yellon, Teasley, et al., 1999; Drazen et al., 2000; Mann et al., 2000; Prendergast et al., 2001; Prendergast et al., 2002; Yellon and Tran, 2002); however, we do not view the majority of data published to date on effects of photoperiod on immunity as adequate tests of immune function (as described above). Rather, immune function is commonly inferred based on measures of the immune system and immune responses. This is analogous to, and comes from the same tradition as, obtaining measures of the reproductive system and inferring reproductive function. Indeed, the only measure that approximated immune function in Zhou et al. was the DTH response—an antigen-specific cell-mediated immune response that mediates beneficial or deleterious aspects of immune function, depending on the nature of the antigen (e.g., resistance to bacteria and fungi vs. allergies and autoimmunity)—which did not change in response to photoperiod in Syrian hamsters but is markedly enhanced under short days in Siberian hamsters (see below) (Bilbo, Drazen, et al., 2002). In light of the comparative data referenced above, the issue of whether day length affects the measures of traits commonly regarded as components of the immune system (spleen weights, cell numbers) and the responsiveness of immune cells to mitogens (lymphocyte blastogenesis) is hardly in question. Whether these differences are of functional significance is an empirical issue.

The conclusion that photoperiod has no effect on immune responses in seasonal mammals is premature. The failure to observe an effect of photoperiod on some measures of immunity does not permit the global conclusion that photoperiod does not affect immune responses. Just as neuroendocrine responses to photoperiod are trait specific (e.g., intermediate photoperiods that inhibit gonadal function do not affect prolactin) (Duncan et al., 1985), the same may be true of the immune system. Thus, in a given species, it does not seem unreasonable to expect to find that some immune responses are photoperiodic (e.g., mitogen-stimulated cytokine secretion in Phodopus) and that others are relatively unresponsive to changes in photoperiod (e.g., NK cell activity). Such observations in no way permit the conclusion that photoperiod has no effect on immune responses for that species, let alone an entire class of vertebrates. Proving the null hypothesis is a difficult task, made more so by abundant contradictory evidence and ambiguous definitions.

As readers of this journal are well aware, timing is everything. While Zhou et al. (2002) was apparently under review, several published reports demonstrated striking effects of photoperiod on immune responses with direct relevance to organismal immune function. For example, we recently reported that symptoms of infection, such as fever, anorexia, and lethargy, are affected by photoperiod (Bilbo, Drazen, et al., 2002). Short days attenuated the organismal response to a simulated infection (in vivo lipopolysaccharide treatment); lower production of IL-6 and IL-1β in short days was associated with a reduced duration of fever and diminished anorexia. Short-day exposure also decreased the ingestion of dietary iron—a nutrient vital to bacterial replication—after infection (Bilbo, Drazen, et al., 2002). These responses reflect an integrated immune response to a pseudoinfection that could improve the probability of survival under energetically challenging winter conditions. Another study (Bilbo, Dhabhar, et al., 2002) evaluated photoperiodic modulation of glucocorticoids and the distribution of blood leukocytes during acute stress in Siberian hamsters. This report also described the influence of photoperiod and acute stress on a delayed-type contact hypersensitivity (DTH) response to dinitrofluorobenzene in the skin (essentially identical to the CH response described by Zhou et al.). Short days increased glucocorticoid concentrations and the absolute number of circulating blood leukocytes, lymphocytes, T-cells, and NK cells. Acute restraint stress prompted trafficking of lymphocytes and monocytes out of the blood, but this redirection of leukocytes occurred much more rapidly in short days. The DTH response was greater under short days, and this effect was augmented by acute stress, likely reflecting more rapid redistribution of leukocytes out of the blood and into the skin in short-day animals (Bilbo, Dhabhar, et al., 2002). Under stressful conditions, it may be beneficial for immune cells to exit the blood and move to primary immune defense areas such as the skin, presumably in preparation for potential injury or infection (Dhabhar and McEwen, 1999). Interestingly, Siberian and Syrian hamsters appear to differ categorically in photoresponsiveness of the DTH response. This species difference highlights the trait specificity of immu-
nological photoresponsiveness, in form and in function.

The many reasons for a failure to replicate research have already been considered in the pages of this journal (e.g., Zatz, 2000). In common with Zhou et al. (2002), we routinely observe lower basal lymphocyte proliferation after exposure to short photoperiods. We are at a loss, however, to explain their failure to see photoperiodic effects on circulating IL-6, DTH, or NK cell activity, measures that we, and others, have observed to vary in response to changes in day length. Several fundamental differences in the measurement of NK cell activity and IL-6 (bioassay vs. enzyme-linked immunosorbant assay (ELISA) and in the method of lipopolysaccharide administration (air pouch vs. intraperitoneal) preclude direct comparisons between analogous data collected on the same species but in different laboratories.

The conjecture by Zhou et al. (2002) with regard to the role of leptin as a neuroendocrine mediator of photoperiodic effects on the immune system appears untenable. Zhou et al. measured immune parameters and immune cell responses in both Siberian and Syrian hamsters. Absent measurement or manipulation, however, they concluded that leptin does not play a role in modulating immunity, based in part on a meta-analysis of work originating in our laboratory. Contrary to their conclusions, our reports describing the role of leptin in short-day-induced alterations in immunity are not inconsistent (Drazen et al., 2000; Drazen et al., 2001). The first of the two articles described the effects of photoperiod on changes in serum leptin concentrations in Siberian hamsters. Humoral immunity (IgG primary antibody production in response to a novel protein) was diminished by exposure to short photoperiods in reproductively photoresponsive hamsters, as well as in those that did not exhibit the modal short-day decreases in testis size and body mass (so-called nonresponders). We concluded that leptin concentrations did not predict immunity. These data in no way conflict with the subsequent article (Drazen et al., 2001), which demonstrated, via chronic leptin infusions, that leptin indirectly enhances IgG production in short-day responders by increasing food intake (nonresponders were not included in this study) (Drazen et al., 2001). Contrary to the statement by Zhou et al., leptin concentrations were indeed reported in Figure 1 of Drazen et al. (2001), indicating that short-day hamsters bearing leptin minipumps had circulating leptin concentrations comparable to those of long-day control hamsters. Some of the nonimmune data from Drazen et al. (2001) contrast with those of one research group (e.g., Klingenspor et al., 1996) and, as addressed in the discussion, may reflect differences in the route of antigen injection and/or the photoperiod regime used. Zhou et al. arrive at a conclusion that contradicts two strong inference tests of the role of leptin in humoral immunity. Given that the role of leptin in immunity was not tested, such a conclusion seems unwarranted.

Zhou et al. (2002) interpreted the observed effects of day length on immune parameters as generalized “effects of photoperiod on metabolism and growth processes that are widely reported in seasonal mammals, leading to differences in cellular physiology” (p. 403), and in light of abundant data indicating effects of photoperiod on immunity, they concluded that photoperiod has no direct effect on immune responses per se in seasonal mammals. This conclusion may hinge on the concept of direct versus indirect control of the immune system. Given that functional photoreceptors have yet to be identified on lymphoid tissues, it is unlikely that the amount of light, per se, to which an animal is exposed can directly affect immunity. Rather, day length likely affects immunity via multiple mechanisms, all of which are indirect in the strictest sense of the term. Potential mechanisms include, as Zhou et al. suggest, seasonal changes in metabolism and growth, as well as actions of melatonin on lymphocytes, photoperiodic changes in gonadal hormone secretion, adrenal hormone secretion, metabolic rate, locomotor activity, sleep, and adiposity, to list but a few, all of which are essentially masking effects of day length. The indirect nature of the mechanism(s) by which photoperiodic changes in physiology impinge on the immune system does not diminish its significance. Physiological dissection of these mechanisms should yield basic insights into how immune function is maintained within a seasonally changing organism.

A null hypothesis that remains to be adequately tested can be posed as a question: Do the net effects of photoperiod on measures of the immune system and immune cell responses extrapolate into meaningful differences in immune function? This is a difficult, but neither impossible nor unimportant, question to address. Tests of functional significance would provide context for data indicating evidence for or against photoperiodic changes in immune measures and responses. Increased responsiveness for a given immune trait may be beneficial to host survival in the
face of one type of challenge and detrimental in the face of another. Ultimately, the immune system must only function adequately over time. Perhaps the net consequence of photoperiodic changes in measures of the immune system and immune responses is to simply maintain the same capacity for immune function year-round (Demas and Nelson, 1996).

REFERENCES


