

Effect of Continuous Darkness on Immune Response (In vivo Assay)

Ikbal Kh. Al-Joofy (Ph.D.)
Thana J. Al-Khishali (M.Sc.)

Summary

Background: Reports denote that changes in day length enhance or suppress components of immune function in several mammalian species. The aim of present experimental study is directed to test the hypothesis deals with the effect of photoperiods on some immune limbs responsiveness.

Materials and Methods: Twenty six male and female BALB/C mice, 5-7 weeks old, 14-18gm weight divided into two groups, test groups (n.=8 mice for each sex) and control groups (n.=5 for each sex). Test groups were kept in a dark room for a month, while control groups were kept in a room where the photoperiod was day light: darkness 12:12hr. All studied groups immunized with 0.2ml (10% sheep red blood cells) on day 4 and 8 of the last 12 days of the experiment. The weight of all animals were measured at the beginning and the end of the experiment. Arthus reaction, delayed type hypersensitivity and serum antibody titer were assessed on day 11 and 12 of program.

Results: Significant increased ($P<0.005$) in body weight, index level of Arthus reaction, delayed type hypersensitivity and serum antibody titer in the test groups in comparison with the control groups.

Conclusion: Data are consistent with the hypothesis that immune parameters are enhanced in short photoperiods or continuous darkness.

Keywords: Photoperiod, immune response

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Introduction:

Several studies showed that changes in day length enhance or suppress components of immune function in individuals of several mammalian species.⁽¹⁾ Both visible light of low energy density (diffuse light and polarized light) increased the number of blast transformed cells even in a lymphocyte culture without PHA, and reduced rosette forming of T-lymphocyte⁽²⁾, also ultraviolet radiation present in sun light is the potent immunosuppressive agent, in addition to being the major cause of non melanoma skin cancer.⁽³⁾

Winter conditions may elevate circulating glucocorticosteroid levels, which can compromise immune function⁽⁴⁾. Both ambient temperature and photoperiod were manipulated to assess their effects on immune parameters and the data are consistent with the hypothesis that immune parameters are enhanced in short days⁽⁴⁾. The mechanisms by which short days alter the components of immune function remain unclear.

So the effect of continuous darkness on some immune limbs responsiveness was a target of our study.

Twenty six male and female BALB/C mice, 5-7 weeks old, 14-18gm weight divided into two groups, test groups (n.=8 mice for each sex) and control groups (n.=5 mice for each sex).

Test groups were kept in a dark room for a month, while control groups were kept in a room where the photoperiod was day light: darkness 12:12 hr.

The two studied groups were injected intraperitoneally with 0.2ml (10% sheep red blood cells) on day 4 and 8 of the last 12 day of the experiment, while the weight of all animals were measured at the beginning and the end of the experiment.

Immunological tests

-Arthus and delayed type hypersensitivity (DTH):

These tests were assessed by observing local reaction on day 11 and 12 after immunization all mice with 10% sheep red blood cells.

Arthus reaction was observed as an increase in footpad swelling 4 hours following challenge with RBCs, while DTH reaction peaks at 18-24 hours⁽⁵⁾

In this test each mouse was challenged by injecting the left hind footpad subcutaneously (s.c.) with 50 μ l of 10% sheep RBCs, and 50 μ l normal saline was injected s.c. into the right hind footpad as a control. Footpad swelling measured as reported in Triolo, et al., (1989)⁽⁶⁾.

* College of Science , Dep. Of Biology , AL_ Mustansiriyah University.

**College of Medicine, Department of Anatomy, Al-Mustansi

-Determination of antibody titer

Samples of blood were obtained from the studied groups on day 31 from retroorbital sinus, serum samples were titrated against sheep RBCs by direct hemagglutination and complement fixation test⁽¹⁾

Statistical analysis

The data was calculated by mean of the students t-test.

Results:

Significant increase ($P < 0.005$) in mean body weight of test groups (male & female) as compared to control groups as clearly shown in table 1

Index level of Arthus and DTH to sheep RBCs is demonstrated in table 2, significant elevation ($P < 0.005$) of the mean index of both Arthus and DTH reactions in test groups as compared to control groups.

Significant increase ($P < 0.005$) in the means of antibody titer in the test groups in comparison to control groups by using direct hemagglutination and complement fixation tests as well presented in table 2.

Statistical analysis of the results denoted significant difference ($P < 0.001$) between the two sexes in all parameters used in this study.

Table-1: Effect of continuous darkness on mice body weight

Study groups	Body weight (gm) Mean \pm S.D		Comparison
	At the beginning	After month	
*Control groups			
Male	14.58 \pm 1.42	25.33 \pm 1.13	***Sig. Sig.
Female	18.75 \pm 0.75	23.83 \pm 1.01	
**Test groups			
Male	15.14 \pm 2.80	24.57 \pm 2.20	Sig. Sig.
Female	16.92 \pm 1.85	22.3 \pm 2.76	

* 5 mice for each sex

** 8 mice for each sex

*** Sig. $P < 0.005$

Table-2: Effect of continuous darkness on some immunological parameters

Parameters	Study groups				Comparison
	*Control groups		** Test groups		
	Male M \pm S.D	Female M \pm S.D	Male M \pm S.D	Female M \pm S.D	
Arthus reaction	0.265 \pm 0.017	0.376 \pm 0.052	0.382 \pm 0.041	0.690 \pm 0.024	***Sig.
Delayed type hypersensitivity	0.223 \pm 0.053	0.225 \pm 0.012	0.346 \pm 0.045	0.365 \pm 0.022	Sig.
Antibody titer					
Hemagglutination	141 \pm 25.61	72 \pm 36.49	160 \pm 55.43	112 \pm 16.00	Sig.
Complement fixation	320 \pm 22.89	256 \pm 12.80	1100 \pm 53.44	656 \pm 37.40	Sig.

* 5 mice for each sex

** 8 mice for each sex

*** $P < 0.005$

comparison between the two sexes is significant $P < 0.001$

Discussion:

Our data are consistent with the hypothesis that immune parameters are enhanced in short days, this was reflected by significant increased ($P < 0.005$) in body weight which has marked stimulating action of growth. On the other hand significant elevated in index levels of Arthus reaction, DTH and antibody responses which denote that there is an enhancement in lymphocytes and macrophages functions which controlled by soluble mediators of immune system like IL-1 and IL-2^(8,9). Stimulation of lymphocyte could be caused by alteration of NADPH metabolism or by a change in the redox potential on the lymphocyte membrane⁽⁸⁾.

Significant difference between the two sexes in all parameters used in this experiment, which may be due to many factors, among which is hormonal factor, and physiological specificity.

Indeed, seasonal changes in immunocompetence are driven primarily by changes in day length, as in Siberian hamsters which are seasonally breeding, photoperiodic rodents that exhibit a constellation of changes in immune function when exposed to short winter like photoperiods⁽¹⁰⁾. Short day exposure sufficient to inhibit reproductive function and gonadal hormone secretion, suppresses phagocytosis, granulocyte number and oxidative burst activity, T-cell dependent humoral immunity and in vitro basal lymphocyte proliferation and lymphocyte IL-1 production⁽¹⁰⁾, however short days also increase natural killer cell cytotoxicity and circulating T-cell and B-cell numbers^(11,12,13). Mechanisms by which short days alter these components of immune function remain largely unknown.

The manner in which these changes are integrated into the host response to a given pathogens may be the more meaningful determinant of whether immune function is considered improved or impaired at short photoperiods.

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