Immunomodulating Response and Circadian Fluctuations of Immunity Parameters in Control Animals

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Effects of immunomodulators and ionizing radiation on 5-nucleotidase activity in macrophages of peritoneal exudate are governed by the mirror symmetry principle, similar to that determining the effects of *Yersinia enterocolitica* on the lymphocyte blastogenesis of inbred mouse splenocytes. The control values are particularly important in comparative experiments.

Key Words: *immunomodulating effect; Wilder's law of initial values; principle of mirror symmetry; biorhythms*

Immunological parameters undergo biorhythmological fluctuations, including circadian ones. It is known that immunomodulating (IM) effect depends on the initial immunological status of the organism; some authors report that IM reactions are also determined by Wilder's law of initial values [1,4].

We investigated the relationship between the IM effect and the initial state of the immune system in intact animals at the moment of drug injection and at the moment of investigation of the effect of immunomodulator (IM). We investigated the conformity of IM response to Wilder's law and the mirror symmetry principle (MSP). Wilder's law reflects the relationship between changes in the studied parameter and its initial value at the moment of exposure. According to this regularity, a function is the less stimulated and easier suppressed, the stronger it is activated at the moment of exposure, and vice versa [4]. Previously we demonstrated a relationship between IM effect and the level of the studied parameter in control animals at the moment of investigation and proved that IM reactions obey MSP [2]. The MSP states that the direction and magnitude of IM effect on a parameter inversely depends on the level of this parameter in control animals at the moment of investigation.

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MATERIALS AND METHODS

Experimental conditions were chosen so that the level of the studied parameter in intact animals was changing considerably. It was attained by injecting immunomodulator at various hours of the day.

Activity of 5'-nucleotidase (5-N) of peritoneal exudate macrophages was the indicator of IM activity of the agents [3].

RESULTS

Macrophagic 5-N activities in BALB/c mice after subcutaneous injection of salmosan and phosphoprenyl in doses of 100 and 4 μg/animal, respectively, are shown in Fig. 1. The drugs were injected in single doses at 6.00, 18.00, and 24.00. Enzyme activity was measured 6, 12, and 18 h postinjection. In intact animals, 5-N activity was measured at the moment of injection (control 1) and at the same terms as in experimental animals (control 2).

There was no correlation between the initial level of 5-N activity at the moment of drug injection and changes in this parameter under the effect of IM (Fig. 1). A clear-cut relationship between enzyme activity in control animals at the moment of investigation and the direction of IM effect was observed for both drugs at all terms postinjection: 5-N activity in experimental

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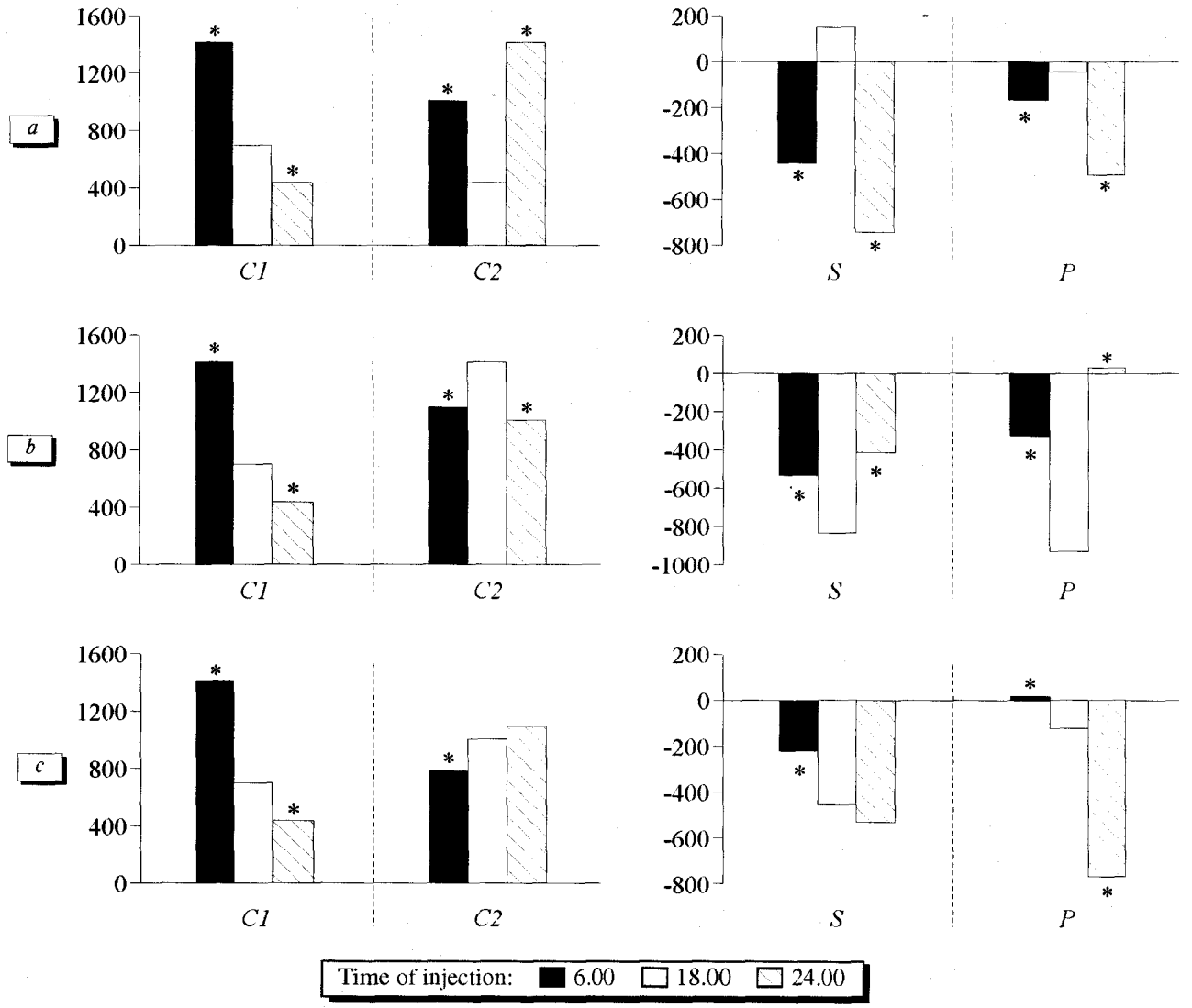

Fig. 1. Activity of 5-nucleotidase of peritoneal exudate macrophages 6 (*a*), 12 (*b*), and 18 (*c*) h after injection of salmosan (S) and phosphoprenyl (P) at various times of the day. Here and in Figs. 2-4: *C1* is control 1 (at the moment of injection, initial level); *C2* is control 2 (at the moment of analysis). Here and in Fig. 2: ordinate: activity of 5-nucleotidase, arb. units (multiscan readings×100/10^6 cells); *$p<0.05$ *vs.* the values at 18.00.

animals decreased when control animals had high activity of the enzyme and increased when the controls had low 5-N activity. Although we failed to detect a relationship between the initial level of the studied parameter in the control group and the IM effect on this parameter, it seems that our findings do not contradict Wilder's law. This law is true mainly for physiological functional loading, while the IM exposure used in our experiment are beyond the physiological range. Presumably, Wilder's law describes the effect of physiological doses of IM.

The conformity of IM effect to Wilder's law described previously [1] is in line with our present findings. In a previous experiment [1] the initial level served as the control at the moment of analysis. Such a situation can occur when the IM effect is evaluated soon postinjection (5-20 min). Under these experimental conditions none of the above principles can be preferred. It is possible that previous results also conform to the MSP [1].

Our present findings indicate that the effect of IM on 5-N activity conforms to MSP. This principle is similar to Wilder's initial value law: the same qualitative and quantitative dependence on the control level: the higher the value in control animals, the more pronounced its drop in experimental animals, and vice versa. However, Wilder's law describes the relationship between changes in a parameter in experimental group and its initial baseline value (at the moment of exposure), while MSP between the former and the value in the control group at the moment of analysis.

The effect of ionizing radiation on 5-N activity (Fig. 2) and the effect of *Y. enterocolitica* on the in-

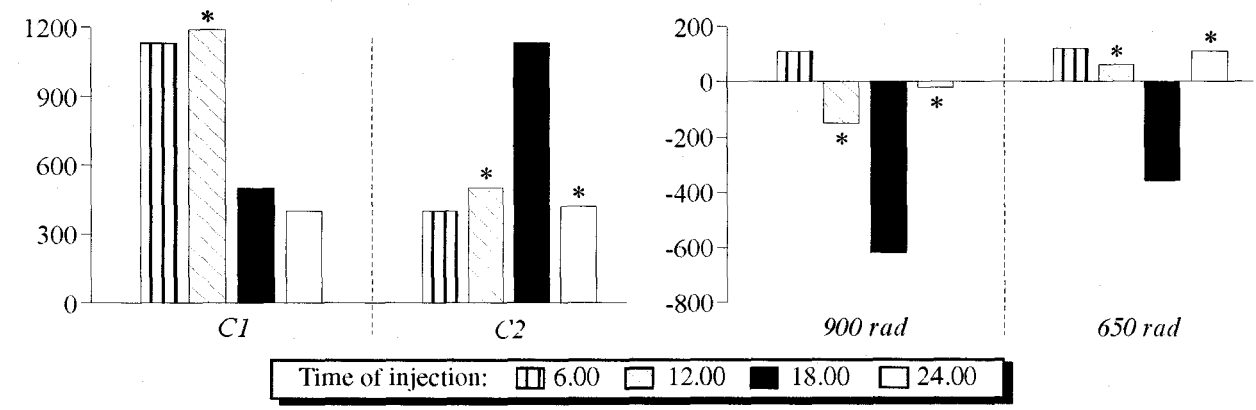

Fig. 2. Activities of 5-nucleotidase of peritoneal exudate macrophages 6 h after irradiation at various time of the day.

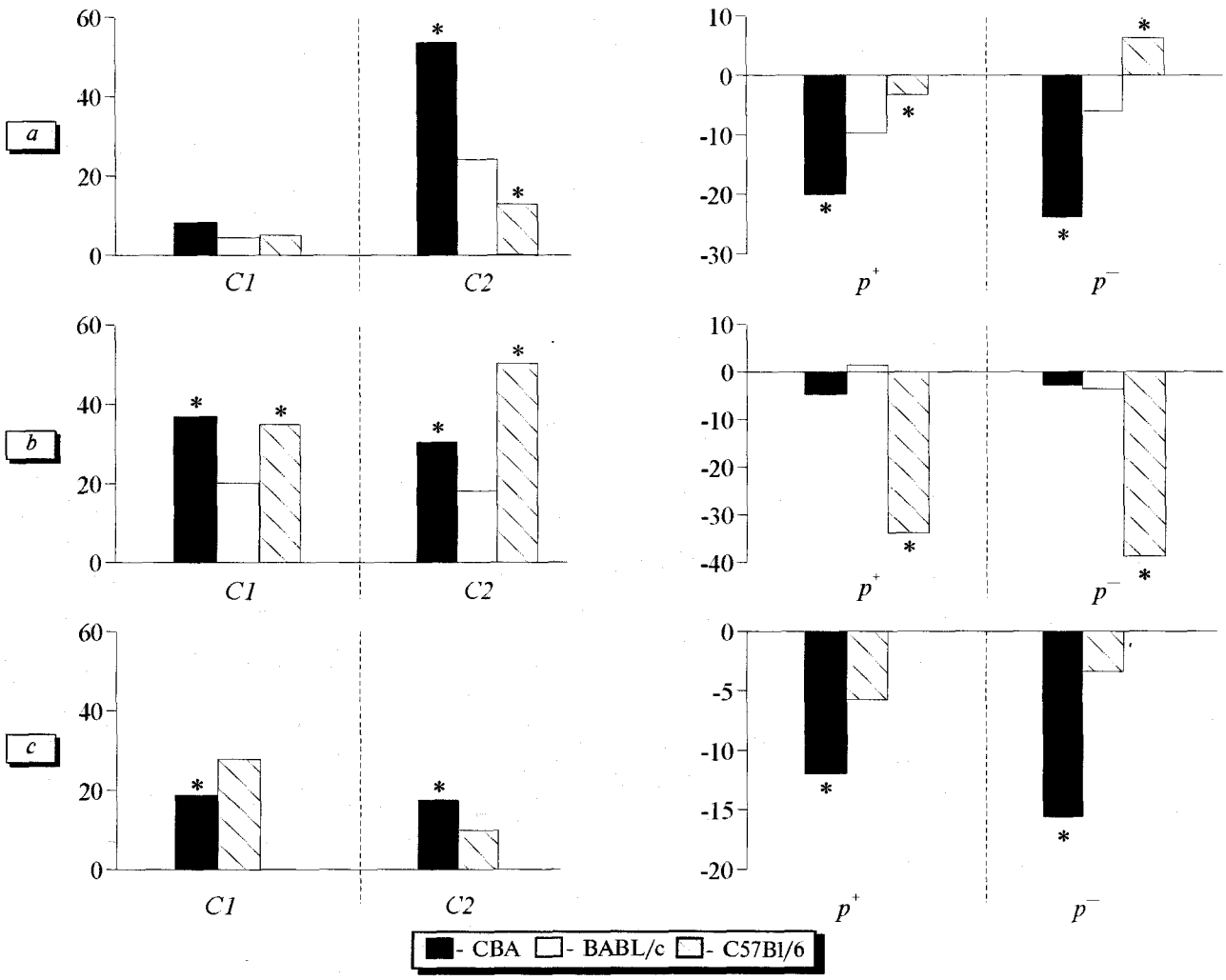

Fig. 3. Changes in spontaneous (*a*) and lipopolysaccharide- (*b*) and concanavalin A (*c*)-stimulated positive (p^+) and negative (p^-) blastogenesis of splenic lymphocytes from different mouse strains under the effect of *Y. enterocolitica*. Time of analysis: *a*) 7 days after challenge with *Y. enterocolitica* at 18.00; *b*) after 3 h at 18.00; *c*) after 3 h at 6.00. $p<0.05$ *vs.* BALB/c mice. Here and in Fig. 4: ordinate in experimental groups: difference between the level of reaction in experimental and control groups, cpm×10^3.

bred mouse splenocyte blastogenesis (Figs. 3 and 4) also conforms to the MSP.

As seen from Figs. 2-4, changes in the studied immunological parameter induced by various factors depend on the level of this parameter in the control at the moment of analysis. This relationship is valid for interstrain differences (Fig. 3) and biorhythmological characteristics of the effect of various modulators on the immune system.

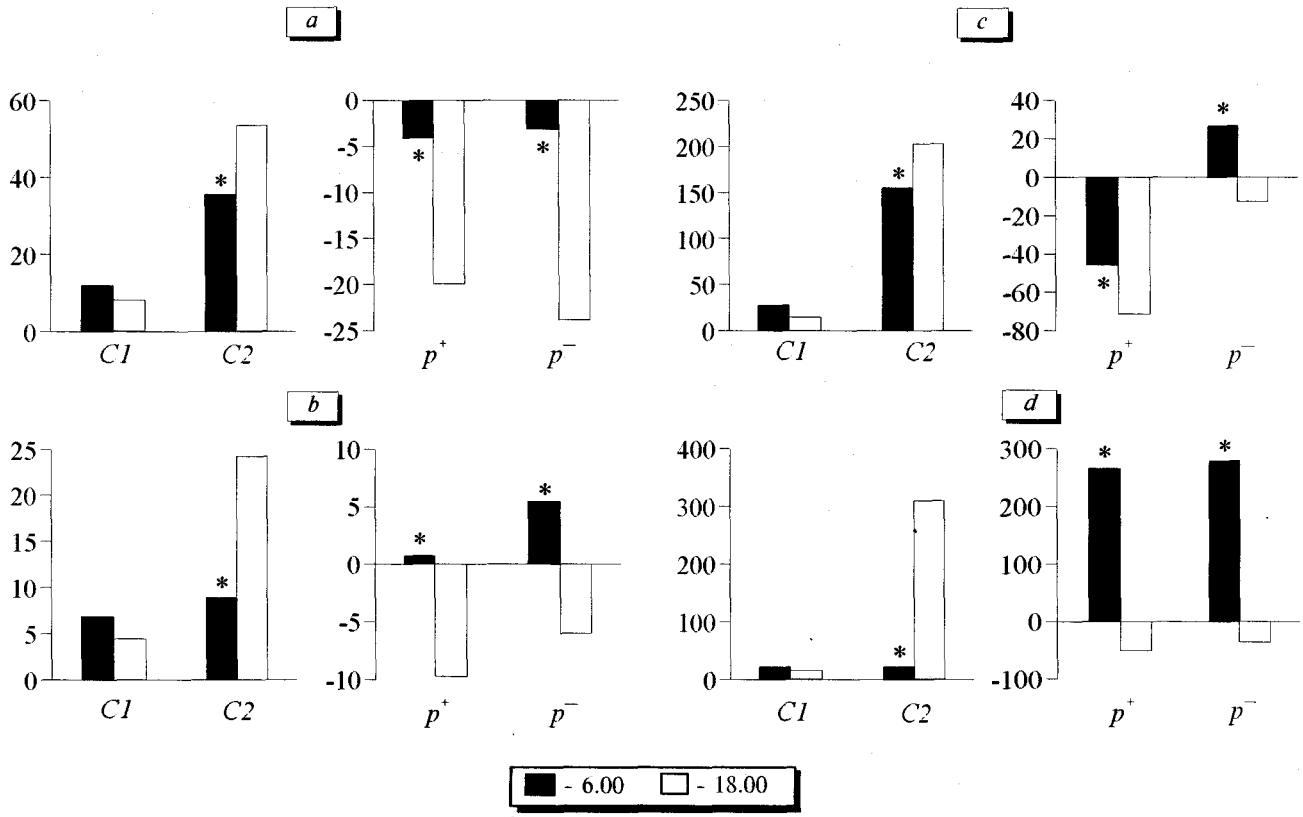

Fig. 4. Changes in spontaneous (*a, b*) and concanavalin A-stimulated (*c*) positive (p^+) and negative (p^-) blastogenesis of splenic lymphocytes from inbred mice under the effect of *Y. enterocolitica* strains injected at different times of the day. *a*) 3 h after injection of *Y. enterocolitica* to C57BL/6 mice; *b-d*) 7 days after injection of *Y. enterocolitica* to CBA (*b*), C57BL/6 (*c*), and BALB/c (*d*) mice. *$p<0.05$ *vs.* analysis at 18.00.

Changes in the absolute values of the control level at all stages of the analysis are usually neglected during interpretation of experimental data, though they should be taken into consideration in all comparative experiments [2]. This will rule out many methodological errors and will give a new insight into the interstrain differences and differences related to biorhythmological characteristics of the immune system.

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