Correlation of Pain Sensitivity and the Humoral Immune Response in Mice during Thermal Stimulation

A. M. Vasilenko, O. G. Yanovskii, O. V. Koptelov, E. E. Metaksa, and L. A. Zakharova

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A hypothesis about a correlation between threshold pain sensitivity and antibody production is proposed and experimentally validated. Immunodeficient mouse strains are characterized by a higher threshold sensitivity to pain than animals with a normal immune response. A highly reliable negative correlation between threshold sensitivity to pain assessed by the hot plate test and the number of antibody-producing cells in the spleen after immunization with sheep red cells is observed in 77% of (CBA×C57Bl/6) F_1 mice examined. The negative correlation is observed both in spontaneous variations of threshold pain sensitivity and during an elevation of this threshold under the effect of preceding nociceptive stimulation.

Key Words: *inbred mice; threshold pain sensitivity; antibody production*

Up to the present time, the relationship between pain and immunity has been regarded mainly from the viewpoint of the effects of nociceptive or other stress-producing stimulation on immunological reactivity. The results of studies indicate that pain can have both depressive and stimulating effects on the immune response [2,11].

Genetically determined characteristics of organization of the endogenous opioid system govern the specific features of nociceptive and defense reactions and the behavior of animals in stress situations, as well as the variants of development of stress-induced and morphine analgesia [3,10,12,14]. The immune response is also a genetically determined reaction [5]. Pure-strain animals are used in studies, the immune response to a certain antigen being the principal criterion for selection. Different forms of immunodeficiency states may be hereditary. For example, C57Bl/6J-bg/bg mice with congenital immunodeficiency due to impaired lysosome function are characterized by a pronounced deficit of morphine analgesia, and this property is transferred by splenic B lymphocytes and macrophages [13].

The existence of a number of common transmitters regulating both immune reactions and pain sensitivity parameters, and the coordinated work of the nervous and immune systems to realize the adaptation process strongly suggest that there is a relationship between threshold pain sensitivity (TPS) and the immune response. The present research was aimed at verifying this hypothesis.

Department of Immune System Transmitters, M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences; Department of Reflexotherapy, N. A. Semashko Medical Stomatological Institute, Moscow. (Presented by R. V. Petrov, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Inbred mice of various haplotypes CBA, C57Bl/6, BALB/c, and MRL/MRL-lpr homozygous for the lymphoproliferation gene lpr (MRL/lpr) and female hybrids (CBA×C57Bl/6) F_1 weighing 18 to 20 g were used in experiments.

TPS was assessed by the hot plate method at 50°C [9]. In some experiments TPS was repeat-

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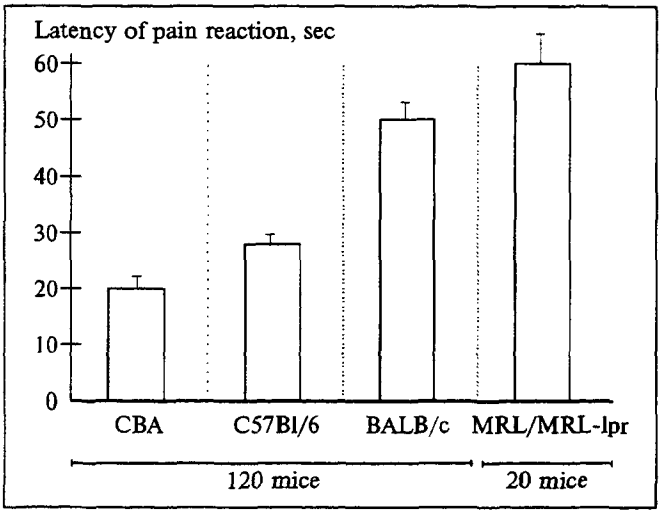

Fig. 1. TPS in mice of different strains.

edly assessed 15 sec and 5, 10, 15, 60, 120, and 180 min after the first test at the same time of day, between 14:00 and 16:00 h. Immediately after the first assessment of TPS or 20, 60, or 180 min after it CBA and (CBA×C57Bl/6) F_1 mice were immunized once with 0.05% sheep red cell suspension (5×10^6) in medium 199 in a dose of 0.5 ml intraperitoneally. The animals were sacrificed on days 4-5 after antigen challenge and the number of antibody-producing cells in the spleen was assessed by a modification of Cunningham's method [4].

The data were statistically processed using Student's and Fisher's tests and Statgraphics analysis of correlations and regressions.

RESULTS

Assessment of TPS in genetically different mouse strains revealed statistically reliable differences in this characteristic. The mean latency of the pain reaction in mice increased in the following order: CBA, C57Bl/6, BALB/c, MRL/MRL-lpr (Fig. 1). It is noteworthy that TPS tended to increase in immunodeficient animals. Analysis of published data showed that the inbred mice used in our experiments were characterized by different degrees of immunocompetence. CBA mice whose TPS is relatively low are highly reactive toward such antigens as sheep red cells, *Yersinia pestis* antigen, *Salmonella typhimurium* 0 and H antigens, and to hapten-carrier conjugates with the trinitrophynyl group used as antigenic determinant, in comparison with the C57Bl/6 mice [5,7,8]. On the other hand, the C57Bl/6 strain is characterized by a higher immunoreactivity to a number of viral infections and by increased interferon production as compared to the BALB/c strain [1]. A higher incidence of spontaneous tumor formation was observed in BALB/c mice with a high TPS (latency of pain reaction 50 sec); moreover, these animals proved to be the most sensitive to radiation [1]. MRL/MRL-lpr mice with genetically determined disorders of the immune system [6] were also found to have a high TPS.

In the next experimental series a correlation analysis of the relationship between TPS and antibody production in individual animals was carried out. (CBA×C57Bl/6) F_1 hybrids were used, which show a greater variation of the examined parameters than pure-strain animals. Since stress-induced analgesia is known to develop within the first 3 h of nociceptive exposure [12], the mice were immunized with sheep red cells 3 h after the first measurement of TPS. The count of antibody-producing cells (APC) in the spleen was assessed on day 5 of the immune response. The variation coefficient for TPS was 33%, and for APC 136%. Correlation analysis failed to reveal a correlation between the latency of the nociceptive reaction and the number of APC (linear correlation coefficient $r=0.07$, $p>0.05$) in the total population (114 mice). The total animal population was therefore divided into 5 groups differing in these characteristics. The data presented in Table 1 demonstrate a reliable

Table 1. Statistical Parameters of Relationship between TPS and APC Count in (CBA×C57Bl/6) F_1 Mice ($M\pm m$)

Group	Number of animals	Latency (x)	C, %	Number of APC per spleen (y)	C, %	Linear correlation coefficient	Linear regression equation
T	114	18.9±0.6	32.8	8 483±1 060	136	−0.07	–
1	33	19.9±0.4	11.1	15 706±2 586*	95	−0.556	$y=91\,116-3\,789x$
2	31	13.4±0.4**	17.2	9 893±1 535	86	−0.555	$y=37\,416-2\,052x$
3	24	15.4±0.5*	16.2	312±44**	70	−0.580	$y=1\,034-51x$
4	18	27.9±1.2**	18.3	1 987±947**	202	+0.556	$y=-10\,311+441x$
5	8	26.3±0.7*	7.9	16 863±3 479	58	+0.11	–

Note. T: total animal population. C: coefficient of variation, determined as the ratio of the mean to the standard deviation. Asterisk: $p<0.01$; two asterisks: $p<0.001$ in comparison with T.

negative correlation between TPS and the APC count in 77% of animals (groups 1, 2, and 3). A reliable positive correlation (r=0.556, p<0.01) was observed in 16% of animals with a high TPS (group 4) and low-level immune response. In 8% of animals (group 5) no reliable relationship between these characteristics could be traced.

Moreover, the groups of mice differed in the trend of TPS changes during repeated assessment of the parameter. In group 1 TPS reliably increased (by 43%) after 15 sec (p<0.05), while in group 2 it increased by 48 and 28% after 15 sec and 5 min, respectively (p<0.01). No changes of TPS were observed in group 3, whereas in group 4 TPS was 38% decreased after 15 sec (p<0.05) and 27% decreased after 5 min (p<0.001).

In contrast to the groups where TPS changed within the first 15 min, the total population developed a 35-96% increase of TPS by the first-third hours (Table 2).

Hence, the time course of TPS differed in the groups of mice we distinguished and differed from that in the total population. We failed to detect changes in TPS in the total population in the same periods, evidently because of the heterogeneity of animals whose reactions might have been oppositely directed, which we did observe in individual groups.

Since nociceptive thermal stimulation causes hypoalgesia, we studied the effect of such an exposure on the development of the immune response. CBA mice were immunized with sheep red cells directly after TPS measurement and 20 min and 1, 3, 6, and 24 h after it. The number of APC in the spleen was counted simultaneously in all groups on day 4 of the immune response. The results are presented in Fig. 2. A reliable drop of antibody production was observed 1, 3, and 6 h after the hot plate test. The maximal, four-fold, reduction of the APC count was observed 1 h after the test.

The data also indicate that the hypoalgesia state goes along with reduced immunological reactivity.

The results of experiments on mice belonging to different strains and first-generation hybrids indicate that an immunodeficiency state or a lowered level of immune response is, as a rule, associated with high TPS values, and a negative correlation between antibody production and TPS is observed in the majority of animals. The phenomenon of combined reduction of pain sensitivity and immunocompetence which we observed is in line with some clinical observations of patients with serious craniocerebral injury, in whom "immune paralysis" developed along with reduced pain sensitivity.

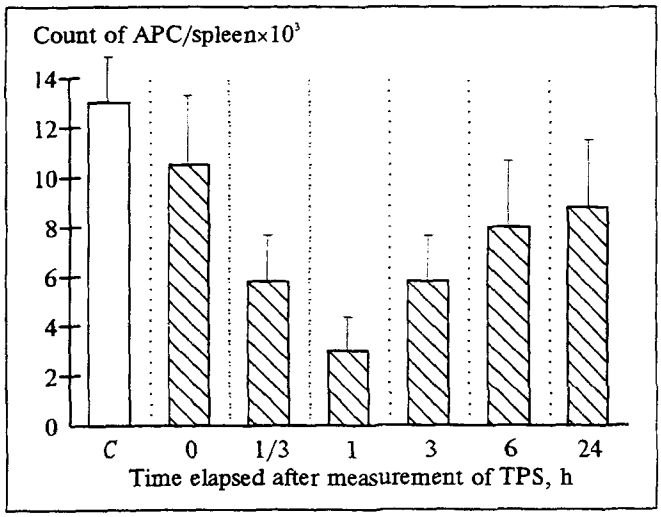

Fig. 2. Suppression of antibody production under the influence of nociceptive thermal stimulation.

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Table 2. Prolongation of Latency of Pain Reaction of Mice in the Hot Plate Test during Repeated Assessment of TPS ($M \pm m$)

Time between 1st and 2nd measurement, h	Latency of pain reaction		Prolongation of latency, %
	1st measurement	2nd measurement	
1	17.8±4.5	24.0±10.0**	34.8
2	16.9±3.7	27.2±11.3**	60.9
3	16.2±5.7	31.8±16.3*	96.3

Note. Asterisk: p<0.05; two asterisks: p<0.01 in comparison with the first measurement.

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Stimulation of Proliferative Activity of Human Natural Killers ($CD16^+CD56^+$ Cells) by Recombinant Interleukin-3 *In Vitro*

S. B. Cheknev

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The proliferative activity of human natural killers ($CD16^+CD56^+$ cells) in the presence of 100 and 1000 IU/ml human recombinant interleukin-3 is investigated *in vitro*. It is shown that recombinant interleukin-3 reliably enhances natural killer proliferation, causing a 9-15.2-fold increase of ^{3}H-thymidine uptake by $CD16^+CD56^+$ cells both in complete culture medium and in conditioned medium. The effect of the factor is 3.9-6.4 and 3.6-8.9-fold more potent than that of recombinant interleukin-2 and granulocyte-macrophage colony-stimulating factor, respectively, in the same doses.

Key words: *natural killers; interleukin-3; proliferation*

Interleukin-2 (IL-2) is regarded as a factor of differentiation for natural killers from bone marrow and peripheral precursors and as a growth factor of mature cells [2,11]. IL-2 can induce the maturation of undifferentiated cell forms into cytotoxic natural killers *in vitro* and the proliferation of human large granular lymphocytes with an increase of the number of $HNK\text{-}1^+$ cells in the culture. However, not all natural killer precursors in the mononuclear cell fraction (MNC) are able to respond to IL-2 action [4], the most potent cells in this case being $T3^-$, Leu 7^-, and $Fc\gamma R^+(^-)$ [4,6]. There are two different populations of precursors of natural killers derived from mouse bone marrow: precursors which can be transformed into cytotoxic cells in the presence of IL-2, and non-transformed cells [12].

Although the fraction enriched with large granular lymphocytes (more than 90%), expresses the maximal proliferative response to IL-2 in an MNC culture [13], the presence of IL-2 alone is not sufficient for latent natural killers to undergo optimal proliferation *in vitro* [15]. It is thought that IL-2 can only support a given proliferation level [15], while other costimulative cytokines are needed for proliferation increase [14]. One such costimulator may be colony-stimulating factor-1 (CSF-1) [5].

The aim of the present study was to investigate the proliferative activity of natural killers ($CD16^+CD56^+$ cells) in the presence of IL-3, which is known as an inhibitor of cellular cytotoxic ac-

Laboratory of Immunochemistry, Gamaleya Institute of Epidemiology and Microbiology, Moscow. (Presented by S. V. Prozorovskii, Member of the Russian Academy of Medical Sciences)

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