

# EFFECT OF BURN TRAUMA ON REACTIVITY OF MOUSE SPLEEN CELLS OF DIFFERENT GENOTYPES IN THE REGIONAL GRAFT VERSUS HOST REACTION

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An important role in the pathogenesis and outcome of burn trauma is played by infectious complications, due to a disturbance of the immunologic reactivity of the victim [12]. Thermal burns are powerful stress factors, and for that reason a special place among responses of the burned individual is occupied by adrenocortical function and the level of endogenous glucocorticoids. Endogenous glucocorticoids can directly influence the control organ of immunity (the thymus) and differentiation of immunocompetent cells. Despite many investigations of endocrine and immune disturbances in burn trauma, interaction between these systems has not been adequately studied. In particular, the effect of individual differences in adrenocortical function on immunologic reactivity in burn trauma has not been investigated. Considering the genetic determination of the glucocorticoid level under normal conditions and during stress [5, 7], in the investigation described below reactivity of lymphocytes of mice of different haplotypes [CBA (H-2<sup>k</sup>) and C57BL/6 (H-2<sup>b</sup>)] with unequal levels of endogenous corticosterone in the local graft versus host reaction (GVHR) after burn trauma was compared.

## EXPERIMENTAL METHOD

Male CBA and C57BL/6 mice and their (CBA × C57BL/6)<sub>F</sub><sub>1</sub> hybrids aged 12-18 weeks, obtained from the "Stolbovaya" inbred animals nursery of the Academy of Medical Sciences of the USSR, were used. The animals were kept under identical conditions of lighting and food. A burn of the IIb-IV degree was inflicted in the middle third of the dorsal region of the animals by means of a spirit flame with an exposure of 7 sec by the method described in [3], through a window measuring 8.7-10 mm<sup>2</sup>, depending on body weight, or 15% of the skin surface. A flame burn was inflicted on the donors of spleen cells (CBA and C57BL/6 mice) between 8 and 9 a.m., under ether anesthesia. The depth of the burn was verified by intravital staining with Evans' blue, by the method of E. V. Gubler. For this purpose, immediately after burning 0.1 ml of a 1% solution of Evans' blue was injected intravenously, the mice were killed 30-40 min later, and incisions were made in the region of the burn. The skin and the most superficial layer of muscle tissue were pale gray in color (necrosis) and the underlying tissues were colored blue, evidence of their viability. Control animals underwent identical manipulations except the flame burn. In the experiments of series I, 6 h and 3 and 7 days after the moment of burn trauma, the spleens were obtained from the donors in order to prepare the cell suspension in medium 199, with a final concentration of  $2 \times 10^8$  nucleated cells/m). In the experiments of series II 5 days before burn trauma, bilateral adrenalectomy was performed on mice of the parental lines. The adrenal glands were removed under ether anesthesia through a midline skin incision in the lumbar region, with division of the muscles in the right and left hypochondria and with approach to the upper poles of the kidneys in order to remove the glands. A mock operation consisted of the above-mentioned manipulations excluding removal of the adrenals. After the operation the animals were given 0.85% sodium chloride solution to drink. In the two series of experiments the immunoreactivity of the splenic lymphocytes was determined by the regional GVHR method, induced in (CBA × C57BL/6)<sub>F</sub><sub>1</sub> hybrids which, for genetic reasons, cannot react to antigens of the par-

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TABLE 1. Induction of Regional GVHR in (CBA × (C57BL/6)F<sub>1</sub> Hybrids by Spleen Cells of CBA and C57BL/6 Mice Subjected to Burn Trauma ( $M \pm m$ )

Line of donors of SC	Number of experiments	Number of mice	Weight increase index of popliteal LN, after injection of SC in a dose of 10 <sup>7</sup> from parental lines of mice into F <sub>1</sub>			Control	p
			6 h after	3 h after	7 days after		
CBA	3	157	5.09±0.17	5.46±0.26	2.71±0.08	3.59±0.16	<0.01
C57BL/6	2	108	3.24±0.22	3.58±0.17	2.42±0.13	4.69±0.30	<0.01

Legend. Here and in Table 2: SC) spleen cells, LN) lymph nodes.

TABLE 2. Effect of Adrenalectomy on Donors on Immunologic Reactivity of Spleen Cells 6 h after Burn Trauma ( $M \pm m$ )

Group	Line	Number of mice	Adrenalectomy	Mock operation	Weight increase index of popliteal LN in (CBA × C57BL/6)F <sub>1</sub> hybrids
1	CBA	15	+	—	3.39±0.18
2	CBA	14	—	+	4.63±0.21*
3	CBA	15	—	—	3.50±0.22
4	C57BL/6	15	+	—	3.70±0.22*
5	C57BL/6	15	—	+	3.24±0.24*
6	C57BL/6	15	—	—	4.28±0.32

Legend. +) Procedure took place, —) procedure did not take place. \* $p < 0.05$  Compared with initial parameters.

ental lines. The GVHR was induced in the popliteal lymph nodes of the experimental F<sub>1</sub> hybrids by injection of 50 μl of a suspension containing 10<sup>7</sup> spleen cells from burned donors, beneath the skin of the right hind foot, by a modified method described previously [11, 8]. An injection of 10<sup>7</sup> spleen cells from donors not subjected to burn trauma was given into the control F<sub>1</sub> hybrids. The experimental and control F<sub>1</sub> hybrids were killed 7 days after induction of the GVHR, the popliteal lymph nodes were removed and dehydrated in acetone, after which they were weighed with an accuracy of 0.01 mg. The degree of development of the regional GVHR was assessed as the increase in weight of the right lymph node compared with the control left lymph node (late increase index). The results were subjected to statistical analysis by Student's *t* test [1].

## EXPERIMENTAL RESULTS

It will be clear from Table 1 that 6 h after burning the ability of spleen cells of CBA mice to induce a local GVHR was increased compared with the control, and this increased ability to induce the GVHR was still present on the 3rd day after burning. A decrease in reactivity of the spleen cells in the local GVHR was recorded 7 days after burn trauma in the CBA mice. In C57BL/6 mice, unlike CBA, the reactivity of the spleen cells in the GVHR was reduced as early as 6 h after burn trauma, with a maximal decrease on the 7th day after trauma. Incidentally, spleen cells from control CBA mice without burns were less able to induce the GVHR than spleen cells of control C57BL/6 mice. Adrenalectomy in the spleen cell donors before infliction of the burn prevented differences in reactivity of the spleen cells of the CBA and C57BL/6 mice 6 h after burning (Table 2, groups 1 and 4), although the ability of C57BL/6 mice, undergoing the mock operation, to induce the GVHR was reduced compared with CBA mice (groups 2 and 5).

CBA mice are distinguished by a higher level of endogenous corticosterone and by greater resistance to stress than C57BL/6 mice, which have a low endogenous corticosterone level [2, 6]. Morphologically, the spleen of CBA mice contains more white pulp than red, by contrast with C57BL/6 mice [6]. The possibility cannot be ruled out that in response to burn trauma the number of reactive lymphocytes in the spleen of the CBA mice remained at a higher level 6 h and 3 days after burning. It may be that in CBA mice, with a high endogenous corticosterone level, more cortical-resistant T lymphocytes differentiate, and these induce the GVHR. Burn trauma in C57BL/6 mice inhibited reactivity of splenic lymphocytes at all times of the investigation, and this can be explained by redistribution of lymphocytes under conditions of stress [10, 13] with their increased emigration from

the spleen into the bone marrow, or with a change in the relative numbers of helper and suppressor T cells in favor of the latter [14]. The largest number of suppressor T cells was recorded in mice 14 days after burning [9].

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