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NONSPECIFIC T-SUPPRESSORS IN EXPERIMENTAL TICK-BORNE ENCEPHALITIS

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Summary. — Suppressor cells inhibiting graft-versus-host reaction under conditions of two-way incompatibility were activated in lymphoid organs of tick-borne encephalitis virus-infected mice. Cells with suppressor activity were found in the thymus, peripheral lymph nodes (LN) and spleen but not in the bone marrow, or peritoneal exudate cells adhering to the plastic surface. The cells were identified as T lymphocytes based on the following properties: sensitivity to anti-theta serum, inability to adhere to plastic surface, and resistance to anti-mouse gamma-globulin serum. The T suppressors were activated 3 days after infection (p. i.) in the thymus and LN and at 4 days in the spleen; they were detectable until the appearance of clinical signs of the disease (8-9 days p. i.).

Key words: tick-borne encephalitis virus; graft-versus-host reaction, T suppressors

Introduction

Experiments with noninfectious antigens yielded numerous data on the existence of populations of immunoregulatory cells (Ataullakhanov *et al.*, 1978; Gershon, 1978; Khaitov and Petrov, 1978). The capacity to enhance or inhibit the immune response in vitro and in vivo has been reported for T and B lymphocytes, macrophages and bone marrow (BM) cells (Petrov and Mikhailova, 1978). Investigations of the function of immunoregulatory cells in virus infections were only started (Tandon *et al.*, 1979).

We are presenting evidence on the appearance in experimental tick-borne encephalitis (TBE) in mice of suppressor cells inhibiting primary immunological recognition local graft-versus-host reaction (GVHR). By all their properties these cells were identified as T lymphocytes.

Materials and Methods

Virus. TBE virus strain Sofin, propagated in the brains of 2-3-day-old suckling mice was inoculated intraperitoneally in doses of 10,000 LD_{50} to mice. The virus was titrated by intracerebral inoculation of suckling mice.

Animals. BALB/c and CBA mice weighing 18—20 g were obtained at the animal farm "Stolbovaya" of the U.S.S.R. Academy of Medical Sciences.

Cells. Splenocytes, LN, thymus, peritoneal exudate (PE) and bone marrow (BM) cells were prepared by routine methods (Mosier, 1967; Semenov *et al.*, 1974; Oliver and Goldstein, 1978).

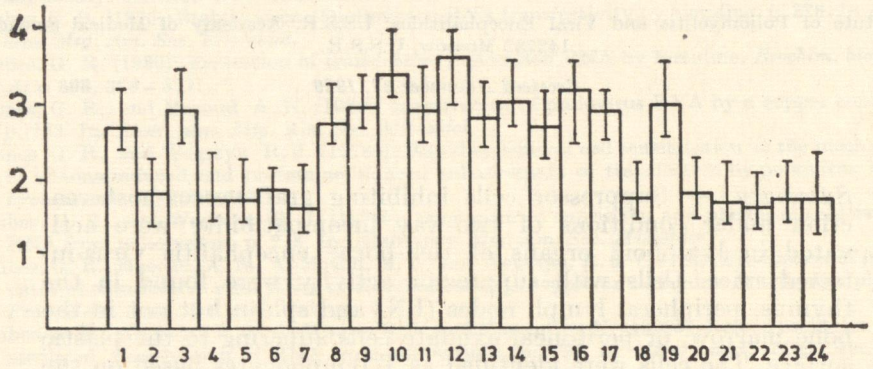

Fig. 1.

Detection of T suppressors in TBE virus-infected mice

Ordinate: GVHR index

Recipients were injected s. c. into the foot pad with:

1 — splenocytes from uninfected donors (SP)
2 — splenocytes from TBE virus-infected donors (SP_{tbe})
3 — lymphocytes from peripheral lymph nodes of uninfected donors (LN)
4 — lymphocytes from peripheral lymph nodes of TBE virus-infected donors (LN_{tbe})
5 — SP + SP_{tbe}
6 — SP + thymocytes from TBE-infected donors (T_{tbe})
7 — SP + LN_{tbe}
8 — SP + bone marrow cells from TBE-infected donors (BM_{tbe})
9 — SP + peritoneal exudate cells (from TBE-infected donors) adhering to plastic surface (PE_{tbe})
10 — SP + SP
11 — SP + thymocytes from uninfected donors (T)
12 — SP + LN
13 — SP + bone marrow cells from uninfected donors (BM)
14 — SP + peritoneal exudate cells (from uninfected donors) adhering to plastic surface (PE)
15 — SP + (SP_{tbe} + AΘS)
16 — SP + (SP_{tbe} + CS)
17 — SP + (T_{tbe} + AΘS)
18 — SP + (T_{tbe} + CS)
19 — SP + (LN_{tbe} + AΘS)
20 — SP + (LN_{tbe} + CS)
21 — SP + (SP_{tbe} + AGGS)
22 — SP + (LN_{tbe} + AGGS)
23 — SP + (T_{tbe} + AGGS)
24 — SP + splenocytes from TBE infected donors, non-adhering to plastic surface

Cells from infected donors were obtained 7 days after inoculation.

Vertical bars indicate confidence limits of the GVHR values shown. The differences were statistically significant ($P \leqq 0.05$) between groups 1 and 2, 5, 6, 7, 16, 18, 20, 21, 22, 23, 24; 2 and 3; 5, 6, 7 and 10, 11, 12. The differences were not significant between ($P \leqq 0.05$) groups 1 and 8, 9, 10, 11, 12, 13, 14, 17, 19; 5 and 16, 21, 24; 6 and 18, 23; 7 and 20, 22.

Table 1. The influence of TBE virus on GVHR under conditions of two-way incompatibility

Virus dose (LD_{50}/0.03 ml)				
10	100	1000	10,000	100,000
2.8(2.3—3.4) / 2.7(2.1—3.2)	2.9(2.6—3.4) / 2.9(2.4—3.6)	2.7(2.4—3.3) / 3.0(2.6—3.7)	3.0(2.4—3.3) / 2.8(2.1—3.4)	3.0(2.6—3.4) / 3.1(2.3—3.8)

Numerator: GVHR index after injection of recipients with splenocytes from uninfected mice suspended in a TBE virus suspension. Denominator: GVHR index after injection of recipients with splenocytes from uninfected animals suspended in appropriately diluted control mouse brain suspension.
In parentheses: lower and upper confidence limits.
GVHR index after injection of recipients with splenocytes from uninfected mice suspended in medium 199 in Hanks' solution was 3.0 (2.4—3.5).

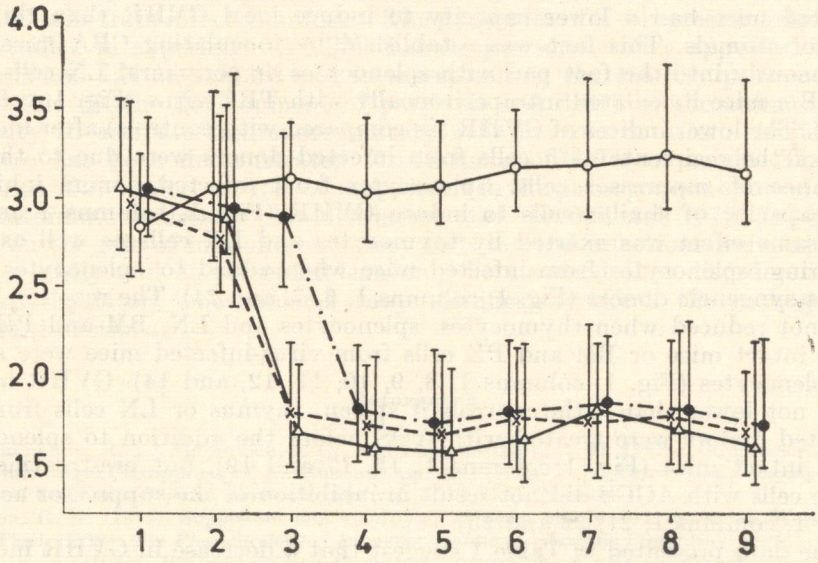

Fig. 2.

Course of T-suppressor activation in TBE virus-infected mice
Recipients (CBA) were injected s.c. into the foot pad with splenocytes of uninfected mice (○) or splenocytes of uninfected mice (BALB/c) mixed with splenocytes (●), LN lymphocytes (×) or thymocytes (△) from infected donors obtained 1, 2, 3, 4, 5, 6, 7, 8, or 9 days after infection. Vertical bars — confidence limits of the GVHR values shown.
Abscissa: days p.i.; ordinate: GVHR index.

Anti-theta-serum (AΘS) was prepared according to Golub (1971) by immunization of rabbits with a brain suspension of BALB/c mice in complete Freund's adjuvant. In a dilution of 1 : 60, AΘS destroyed 92 % thymocytes, 32 % splenocytes, 5 % BM cells and 61 % LN cells in the presence of complement. Cells carrying immunoglobulins on their surface were eliminated by rabbit anti-mouse gamma-globulin serum (AGGS) (Serva) in the presence of complement. Local GVHR under conditions of two-way incompatibility (Zschiesche *et al.*, 1976) was used for the evaluation of cellular immunity. BALB/c mice were donors of immunocompetent cells and CBA mice were the recipients. The donors were injected subcutaneously into the right foot pad with 1×10^7 cells/0.1 ml medium 199 in Hanks' solution. The index of the reaction was calculated from the ratio of the weights of right: left collateral popliteal lymph nodes. The activity of the suppressors was assessed by injecting the recipients into the foot pads with a 1 : 1 mixture of splenocytes from intact and infected mice. In some experiments the cells were pre-treated with AΘS or AGGS. The presence of suppressors was evaluated based on a decrease in the GVHR index of splenocytes of uninfected animals to which cells from infected mice were added as compared to the control group receiving only splenocytes from intact mice. The results were evaluated statistically by Student's test.

Results

The data summarized in Fig. 1 indicate that immunocompetent cells of infected mice had a lower capacity to induce local GVHR than those of control animals. This fact was established by inoculating CBA mice subcutaneously into the foot pad with splenocytes or peripheral LN cells from BALB/c mice inoculated intraperitoneally with TBE virus (Fig. 1, columns 1—4). The lower indices of GVHR (as compared with controls) after inoculation of the recipients with cells from infected donors were due to the appearance of suppressor cells. Splenocytes from infected donors inhibited the capacity of similar cells to induce GVHR (Fig. 1, columns 1 and 5). The same effect was exerted by thymocytes and LN cells as well as non-adhering splenocytes from infected mice when added to splenocytes from intact syngeneic donors (Fig. 1, columns 1, 6, 7, and 24). The reaction index was not reduced when thymocytes, splenocytes and LN, BM and PE cells from intact mice or BM and PE cells from virus-infected mice were added to splenocytes (Fig. 1, columns 1, 8, 9, 10, 11, 12, and 14). GVHR indices were not lower than in the controls if spleen, thymus or LN cells from the infected donors were treated with AΘS before the addition to splenocytes from intact mice (Fig. 1 columns 1, 15, 17 and 19), but pre-treatment of these cells with AGGS did not result in inhibition of the suppressor activity (Fig. 1, columns 1, 21, 22 and 23).

The data presented in Table 1 suggest that a decrease in GVHR index of splenocytes from intact animals after the addition to them of immunocompetent cells from infected donors was not due to the effect of virus present in the latter. The recipients were given spleen cells suspended in a virus suspension, or appropriately diluted intact mouse brain suspension, or medium 199 in Hanks' solution. The GVHR values in the three groups were not significantly different ($P \leq 0.05$).

Fig. 2. illustrates the dynamics of the appearance of suppressor cells in populations of spleen, thymus and LN cells from infected animals. In the

thymus and lymph nodes the suppressor activity appeared 3 days p. i. and in the spleen at 4 days and was detectable till the appearance of clinical signs of infection in the animals (9 days p. i.).

Discussion

The present experiments demonstrated the appearance of suppressors in populations of spleen thymus and peripheral LN cells of mice experimentally infected with TBE virus. The function of the suppressors was detected by local GVHR under conditions of two-way incompatibility. The addition to splenocytes from intact animals of thymocytes, LN cells or splenocytes from infected mice was accompanied by a decrease in the reaction index as compared with the control group which received only spleen cells from intact donors. By their properties like sensitivity to AΘS, inability to adhere to the plastic surface and resistance to AGGS these suppressors were identified as T-lymphocytes. No suppressor activity was observed in thymocytes and LN, spleen, BM, and PE cells from intact mice and in BM and PE cells from infected animals. The dynamics of T-suppressor activation was studied in experimental TBE. The suppressor activity appeared in the thymus and LN on the 3rd and in the spleen on the 4th day p. i. and was detectable till the development of paralysis and death of the animals. The capacity to activate immunoregulatory cells in vivo or after stimulation in vitro has been demonstrated for antigens of different origin. There is evidence of an important role of suppressors in the pathogenesis of oncological and autoimmune diseases (Ataullakhanov *et al.* 1970; Tatal 1978). Investigations on the role of immunoregulatory cells in viral infections were initiated only recently and at present it can only be assumed that disorders in the immunoregulatory mechanisms may significantly affect the pathogenesis of viral infections.

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