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SERUM FACTOR BLOCKING THE ACTIVITY OF VIRUS-INDUCED AUTOREACTIVE T LYMPHOCYTES OF MICE

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Summary. — Normal mouse serum was found to contain a factor blocking in vivo the effect of autoreactive cells accumulating in spleens of mice infected with tick-borne encephalitis (TBE), Langat, dengue type 2 (D2), and attenuated yellow fever (17D strain) viruses. The activity of the factor was manifested only, when the autoreactive lymphocytes had H-2 antigens identical with H-2 antigens of the serum donors. The hypothesis is discussed that serum factor protects the host against clones of autoreactive T lymphocytes generated in viral infections.

Key words: *immunoregulation*; *serum factor*; *autoreactive T lymphocytes*; *flavivirus infections*

Introduction

According to the currently held concepts, among lymphocytes of healthy individuals there are always subpopulations capable of recognizing self antigens as non-self ones. Such lymphocytes designated as "autoreactive" are considered to represent one of the mechanisms of autoimmune process (Konler and Rowley, 1977; Cooke and Lydyard, 1981). The population of the autoreactive cells increases significantly in viral infections (Pfiizenmaier *et al.*, 1975; Proffit *et al.*, 1977). Despite this, however, the clinically manifest consequences of the autoimmune response are far from being omnipresent which is explained by the existence of control mechanisms including immunoregulatory cells and blocking factors (Cohen and Wekerly, 1977; Talal, 1978).

In the currently available literature the role of the blocking factor(s) in the pathogenesis of conventional viral diseases has not been dealt with sufficiently, although there are reports on its detection in patients with subacute sclerosing panencephalitis and infectious mononucleosis (Swick *et al.*, 1976; Veltry *et al.*, 1981). This paper presents the evidence demonstrating the existence in normal mouse serum of a factor blocking in vivo the activity of autoreactive cells induced by flaviviruses. The H-2 restriction effect of this factor has been demonstrated.

Materials and Methods

Viruses. Tick-borne encephalitis (TBE, the Sophin strain), Langat (TP-21 strain), D2 (strain No. 23085), and the vaccine (17D) strain of yellow fever virus were propagated in the brains of 2 to 3-day-old mice and inoculated intraperitoneally in 10,000 LD_{50} into experimental animals. The viruses were titrated in suckling mice inoculated intracerebrally with serial dilutions of brain suspensions.

Mice. Strains BALB/c (H-2^d), CBA (H-2^k), C57BL (H-2^b), AKR (H-2^k), DBA (H-2^d), hybrids of the first generation F_1 (CBA × C57BL) (H-2^{kb}) and F_1 (CBA × BALB/c) (H-2^{kd}) weighing 16 – 18 g were obtained from the U.S.S.R. AMS farm "Stolbovaya". The method for producing splenocytes, thymocytes, or lymph node cells has been reported elsewhere (Semenov *et al.*, 1974). Mouse blood serum was prepared as follows: the blood collected from the retroorbital sinus of uninfected mice was incubated for 1 hr at 37 °C and 2 hr at 4 °C followed by centrifugation at 1500 rev/min for 10 min to remove blood cells and clot. Freshly prepared serum without signs of haemolysis was used in the experiments.

Experimental design. The activity of autoreactive T lymphocytes was assessed by means of the local graft versus host reaction (GVHR). Intact recipients were inoculated subcutaneously into the right foot pad with 1×10^7 syngeneic splenocytes of virus-infected mice 0.1 ml of medium 199 in Hanks' solution. Splenocytes of the infected donors were harvested on day 7 post infection (p.i.). The index of local GVHR was calculated from the ratio of weights of the right/left popliteal lymph nodes. As an additional control, the recipients were inoculated into the foot pad with splenocytes from syngeneic uninfected mice given 7 days before the adoptive transfer an appropriate dilution of uninfected suckling mouse brain. For detection of the serum blocking factor, splenocytes of the infected donors were treated with the normal mouse serum under study for 1 hr at 4 °C using 1×10^8 cells/ml. After contact with serum, the cells were washed twice with medium 199 in Hanks' solution, diluted in the fresh medium and tested in GVHR as described above. A decrease in the index of GVHR induced by mouse serum-treated splenocytes as compared with intact cells was regarded as an indicator of the presence of the serum blocking factor. The activity of supressors in uninfected serum donors was studied by inoculation of the recipients subcutaneously into the foot pad with a mixture of autoreactive cells and cells of lymph nodes or thymus of tested donors according to the method described previously (Khozinsky and Semenov, 1980).

In some experiments, the possibility of induction by the autoreactive cells of systemic GVHR in syngeneic recipients was studied. For this purpose, intact recipients were inoculated intravenously with 5×10^7/0.3 ml splenocytes of the infected donors suspended in medium 199 in Hanks' solution. In these animals on days 8, 10, 12, 14, and 16 after the adoptive transfer the weights of the liver, spleen, and lymph nodes were determined and compared with those from intact mice or recipients inoculated intravenously with splenocytes of uninfected donors. The results were treated statistically using Student's criterion.

Results

Serum of normal CBA mice can block in vivo the effect of autoreactive lymphocytes accumulating in the spleen of mice infected with flaviviruses. As shown in Table 1, splenocytes of the infected donors induced no local GVHR if they had been inoculated into the foot pad of syngeneic recipient after preincubation with serum from syngeneic but not allogeneic animals. Normal serum from CBA mice blocked the activity of autoreactive cells induced in mice of the same strain by different members of the *Flavivirus* genus: both by those inducing an acute disease (TBE virus) and by the causative agents of asymptomatic infection (Langat, D2, and 17D yellow fever virus).

Our experiments revealed the dependence of the blocking effect of the serum upon the availability of a common H-2 haplotype in donors of this serum and donors of autoreactive lymphocytes (Table 2). Thus, serum

Table 1. Detection of serum factor inhibiting the activity of virus-induced autoreactive splenocytes*

Group	Treatment of splenocytes with	Autoreactive splenocytes** induced in CBA mice infected with				Splenocytes of syngeneic intact donors
		TBE	Langat	yellow fever	D2	
1	Normal CBA mouse serum	1.2***	1.1	1.0	1.0	1.1
2	Normal BALB/c mouse serum	2.0	2.0	1.9	1.8	1.1
3	Medium 199 in Hanks' solution	2.2	2.1	1.8	1.9	1.0

* Intact recipients of CBA (H-2^{kk}) strain were inoculated into the foot pad with 1×10^7/0.1 ml of syngeneic autoreactive splenocytes pre-treated in vitro with mouse serum for 1 hr at 4 °C.

** Autoreactive splenocytes were obtained at 7 days post inoculation.

*** Differences are statistically significant between GVHR values in mice groups 1, 2, and 3 receiving splenocytes from infected donors ($\leqq 0.05$). GVHR values in recipients of autoreactive cells significantly differ from those in mice inoculated with splenocytes of syngeneic intact donors in groups 2 and 3 ($P \leqq 0.05$). No differences have been found between similar values in the group 1 ($P > 0.05$).

Table 2. H-2 restriction of the blocking effect of normal animal sera operating in syngeneic system of GVHR induced by splenocytes from TBE virus-infected mice

Group	Recipient*	Donor	Splenocytes treated with sera of mice							Control treatment
			CBA H-2^{kk}	C57BL H-2^{bb}	AKR H-2^{kk}	BALB/c H-2^{dd}	DBA H-2^{dd}	F_1 (CBA × C57BL) H-2^{kb}	F_1 (CBA × BALB/c) H-2^{kd}	
1	CBA	CBA	1.0**	2.0	1.2**	2.1	2.0	1.1**	1.2**	2.1
2	BALB/c	BALB/c	2.1	2.0	2.1	1.0**	1.1**	2.1	1.2**	2.2
3	CBA	BALB/c	2.5	2.3	2.4	n. d.	n. d.	n. d.	n. d.	2.3
4	BALB/c	CBA	n. d.	n. d.	n. d.	2.3	2.2	n. d.	n. d.	2.1

* Intact recipients were inoculated into the foot pad with 1×10^7 syngeneic splenocytes of infected donors in vitro pre-treated either with normal mouse serum (from corresponding strains) or with medium 199 in Hanks' solution (control treatment).

** The values of GVHR index marked by asterisks differ significantly from the rest values of the same group ($P \leqq 0.05$).

n. d. = not done

from DBA mice (H-2^{dd}) blocked the capacity of splenocytes obtained from TBE virus-infected BALB/c (H-2^{dd}) mice to induce local GVHR in syngeneic recipients. Studies with sera from the first generation hybrids showed partial identity of H-2 antigens to be sufficient for the development of the blocking effect.

The blocking effect of normal serum was not found to be associated with destruction of splenocytes. The number of viable spleen cells after treatment with syngeneic serum did not change as compared with that in control consisting of the same cells suspended in medium 199 in Hanks' solution. Besides, syngeneic serum did not affect the intensity of GVHR induced by these cells under conditions of bilateral incompatibility (Table 2, groups 3 and 4). Proceeding from the current concepts on regulation of immune response one could assume that the discovered blocking factor is a mediator of non-specific suppressor cells present in normal mice. In order to check this assumption, the autoreactive cells induced by the viruses under study were mixed with equal amounts of splenocytes, lymph node cells or thymocytes from uninfected syngeneic donors, and then the mixture was examined for its capacity to induce local GVHR. No non-specific suppressors were found in any of the immunocompetent cell populations tested. In discussions on the role of the discovered serum factor in the pathogenesis of viral infections there naturally arose the question of its influence on the circulating autoreactive cells. In this connection the following experiments were carried out. Splenocytes from TBE or Langat virus-infected mice were inoculated intravenously or subcutaneously into the foot pad of normal mice. As expected, in the latter case a local autoimmune process developed: splenocytes of the infected mice induced GVHR in syngeneic recipients. At the same time, after inoculation of the autoreactive cells into the blood stream no systemic GVHR developed: the weight of the liver, spleen, and lymph nodes remained within normal limits for 16 days(the observation period) as compared with controls. It may be assumed, consequently, that the serum factor protects the infected animals against the action of circulating virus-induced autoreactive cells.

Discussion

The results of the present study show that serum of normal mice had a factor(s) blocking in vivo the activity of virus-induced autoreactive lymphocytes previously identified as T cells (Vargin and Semenov, 1980; Khozinsky and Semenov, 1982). The effect of this factor is not associated with destruction of T lymphocytes. It is not a product secreted by non-specific suppressor cells. Apparently it should be classified into the category of blocking factors — extracellular substances inhibiting lymphocyte activity by interaction (reversible blocking) with antigen-specific receptors (Cohen and Wekerle, 1977).

The question whether serum factor of mice is a histocompatibility self-antigen, an autoantibody directed either against this antigen or against complexes of self antigen with autoantibody or whether it represents antiidiotype antibody remains open so far.

It has first been established that the effect of the blocking serum factor follows the rule of H-2 restriction. The activity of lymphocytes recognizing self antigens as non-self is blocked only when they have H-2 antigens identical with those of serum donors. In our opinion, the presence of the blocking factor alongside with previously described T suppressors prevents the development of clinically manifest autoimmune syndrome in most viral infections of mice (Khozinsky and Semenov, 1980). This assumption has been confirmed by the results of a number of experiments. Virus-induced autoreactive splenocytes induce GVHR only when inoculated subcutaneously into the foot pad. After intravenous inoculation of these cells no signs of systemic GVHR was observed probably due to the action of the circulating blocking factor. If this hypothesis is true, the concentration of the blocking factor(s) should be low in the serum of New Zealand mice.

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