Acta virol. **28**: 212—217, 1984

IN VIVO INHIBITION BY INTACT MOUSE SERUM OF THE ACTIVITY OF THE FLAVIVIRUS-INDUCED T-SUPPRESSORS OF AUTOREACTIVE T-LYMPHOCYTES

V. V. KHOZINSKY, B. F. SEMENOV

Institute of Poliomyelitis and Viral Encephalitides.
Academy of Medical Sciences of the U.S.S.R., 142782 Moscow, U.S.S.R.

Received July 8, 1983

Summary. — In the serum of healthy mice a factor was found inhibiting the in vivo effect of T-suppressors of autoreactive T-lymphocytes (TS_{artl}) induced in mice by tick-borne encephalitis (TBE) virus, Langat virus, dengue virus type 2 (D2) and attenuated strain 17D of yellow fever virus. Activity of the serum factor (s) was demonstrated providing that the TS_{artl} had H-2 antigens completely or partially identical with the H-2 antigens of serum donors. The activity was not connected with destruction of TS_{artl}. The factor (s) did not inhibit the in vivo activity of TBE virus-induced T-suppressors directed to the effectors of graft versus host reaction (GVHR) in allogeneic mice. These suppressors differed from TS_{artl} in their distribution in the organs of immune system. As suggested, the serum factor(s) inhibiting TS_{artl} may be involved in pathogenesis of virus infections and in regulation of virus-induced autoimmune processes.

Key words: immune regulation; serum factor; T-suppressors; autoreactive T-lymphocytes; flaviviruses

Introduction

At present, the majority of specialists suggest in the population of immunocompetent cells of healthy organism the existence of precursors of autoreactive T-lymphocytes (ARTL), which are capable to distinguish between self and genetically foreign antigens (Cohen and Wekerle, 1977; Wekerle, 1978; Cooke and Lydyard, 1981). However, activity of these cells is not usually manifested due to the complex system of regulation, including different population of immunoregulatory cells and their products, antiidiotype antibodies, blocking factors, etc. An appearance of functionally active autoreactive cells is considered to result from disturbance of the immunoregulatory system (Jerne, 1973; Cunningham, 1976; Cohen and Wekerle, 1977; Talal *et al.*, 1980). Though the appearence of autoreactive lymphocytes and antigen-specific suppressors inhibiting the immune response to heterologous antigens was observed in many virus infections, the regulation mechanism

of the effectors of autoimmune reactions in virus-caused diseases remains less clear (Levy, 1974; Denman *et al.*, 1976; Semenov *et al.*, 1982).

In our previous studies we demonstrated that the activity of virus-induced ARTL eliciting GVHR in syngeneic recipients was controlled by TS_{artl}, which were activated by virus infection, as well as by serum factor(s) present in serum of intact mice possessing H-2 antigens identical with those of ARTL donor mice (Khozinsky and Semenov, 1980; 1983). This paper presents data on a serum factor inhibiting the activity of virus-induced TS_{artl} and on the heterogeneity of virus-induced antigen-nonspecific T-suppressors populations.

Materials and Methods

Viruses. Strain Sophin of TBE virus, strain TP-21 of Langat virus, strain No. 23085 of D2 virus and vaccine strain 17D of yellow fever virus were grown in the brain of 2 to 3-day-old mice and titrated on suckling mice inoculated intracerebrally with serial dilutions of infectious brain suspension.

Inbred mice from the breed „Stolbovaya" (Acad. Med. Sci. of the U.S.S.R.) weighing 16—18 g were inoculated intraperitoneally with 0.3 ml vol. of medium 199 in Hank's balanced solution (HBSS) containing 10^5 LD_{50} of the virus suspension. The lines BALB/c, CBA, C57BL, DBA and AKR or their F_1 hybrids (CBA × C57B1) and (BALB/cxC57B1) were employed; in each series of experiments animals of the same sex were used.

The mouse serum was prepared as follows: the blood collected from retroorbital sinus of intact, uninfected mice, was incubated for 1 hr at 37 °C followed by 2 hr incubation at 4 °C and subsequent centrifugation to discard blood cells and clots. Only non-haemolytic serum was used in further experiments.

Preparation of splenocytes, thymocytes and lymph node cells was described previously (Semenov *et al.*, 1974). The cells were collected from mice on day 7 post-infection (p.i.) with a given virus; splenocytes served as the source of ARTL and thymocytes or lymph node cells as the source of TS_{artl}, respectively (Khozinsky and Semenov, 1980).

Evaluation of the effect of suppressor cells and serum factor. Uninfected recipients were inoculated into footpad with 0.1 ml volumes of medium 199 in HBSS containing 10^7 ARTL alone or their mixture with the same amount of syngeneic thymocytes or lymph node cells from virus-infected donors. The index of GVHR was calculated as the ratio of the mass of isolated collateral lymph nodes from the inoculated and control leg at 6 days after footpad inoculation of the cells. Lower intensity of GVHR in the recipients inoculated with mixture of ARTL and thymocytes or lymph node cells indicated the presence of suppressor (TS_{artl}) cells among the latter cell populations of virus-infected donors. To determine the effect of a serum factor on the activity of TS_{artl} prior to the mixing with ARTL, the lymphocytes were incubated with serum of intact mice (10^7 cells/0.1 ml of the serum) for 1 hr at 4 °C and washed twice with 5 ml of medium 199 in HBSS.

Immmune aggression of allogeneic lymphocytes against their recipients was evaluated using the local GVHR (Khozinsky and Semenov, 1981). Uninfected recipients were inoculated subcutaneously with 0.1 ml volumes of medium 199 in HBSS containing 10^7 splenocytes from allogeneic uninfected donors. As the source of T-suppressors inhibiting the activity of GVHR effectors on the allogeneic system (TS_{gvhr}) served splenocytes, thymocytes or lymph node cells from virus-infected donors collected on day 7 p.i., which were syngeneic in relation to the GVHR effectors Determination of TS_{gvhr} activity and of GVHR indices was the same as in case of ARTL and TS_{artl}, in all experiments keeping the ratio 1 : 1 between investigated GVHR effectors and TS_{gvhr} on one hand or ARTL and TS_{artl} on the other hand. As additional control of TS_{arte} and TS_{gvhr} corresponding populations of immunocompetent cells from uninfected donors were used, displaying no suppressive activity.

Mitomycin C (Serva) in the concentration of 50 μg/ml in medium 199 in HBSS was incubated with immunocompetent cells for 40 min at 37 °C, which were then three times washed by a fresh medium, to determine the effect of mitomycin on the activity of TS_{artl} and TS_{gvhr}, respectively.

Statistical analysis of the results obtained was carried out using the Student's test. Only $p \leq 0.05$ values were considered for statistically significant.

Table 1. Demonstration of the ability of intact mouse serum to inhibit the activity of TBE virus-induced TS_{artl} and of its H-2 restriction

Group of mice	Serum donors for TS_{artl} treatment	Index of GVHR* in the recipients of ARTL and TS_{artl}	
		BALB/c (H-2^{dd})	CBA (H-2^{kk})
1	No TS_{artl} added	2.1	2.2
2	Serum-untreated TS_{artl}	1.4	1.3
3	BALB/c (H-2^{dd})	2.2	1.4
4	CBA (H-2^{kk})	1.4	2.2
5	C57B1 (H-2^{bb})	1.3	1.2
6	AKR (H-2^{kk})	1.4	2.1
7	DBA (H-2^{dd})	2.0	1.4
8	F_1 (CBAxC57B1) (H-2^{kb})	1.3	1.9
9	F_1 (BALB/cxC57B1) (H-2^{db})	2.0	1.4

* Statistically significant differences ($p \leq 0.05$) between groups 1, 3, 7, 9, and 2, 4, 5, 6, 8 for BALB/c (H-2^{dd}) mice and between groups 1, 4, 6, 8 and 2, 3, 5, 7, 9 for CBA (H-2^{kk}) mice, respectively.

Results

As shown in Table 1, the serum from intact mice inhibited the suppressive activity of TBE virus-induced TS_{artl} against TBE virus infection-activated ARTL. The inhibitory effect of the serum was H-2 restricted, i.e. it appeared only in case of common specificities of H-2 gene complex of both TS_{artl} and serum donors.

Thus, activity of TS_{artl} from BALB/c (H-2^{dd}) mice was inhibited by homologous BALB/c (H-2^{dd}) serum as well as by sera from DBA (H-2^{dd}) or F_1 (BALB/cxC57B1) (H-2^{db}) mice, but not by sera of CBA (H-2^{kk}), C57B1 (H-2^{bb}) and AKR (H-2^{kk}) or F_1 (CBA $\times$ C57B1) (H-2^{kb}) mouse donors. Similarly, TS_{artl} from CBA (H-2^{kk}) donors were blocked by sera of CBA (H-2^{kk}) and AKR (H-2^{kk}) mouse lines or F_1 (CBA $\times$ C57B1) (H-2^{kb}) hybrids,

Table 2. The effect of normal mouse serum on TS_{gvhr}

Group of mice	Splenocytes	Donors of thymocytes (TS_{gvhr})	Normal serum	Recipients	Index of GVHR*
1	CBA (H-2^{kk})	—**	—**	BALB/c	2.9
2	CBA	CBA	—	BALB/c	1.7
3	CBA	CBA	CBA	BALB/c	1.9
4	BALB/c (H-2^{dd})	—	—	CBA	3.2
5	BALB/c	BALB/c	—	CBA	1.9
6	BALB/c	BALB/c	BALB/c	CBA	1.8
7	BALB/c	—	—	C57B1 (H-2^{bb})	3.3
8	BALB/c	BALB/c	—	C57B1	1.8
9	BALB/c	BALB/c	BALB/c	C57B1	1.7

* Statistically significant differences ($p \leq 0.05$) for values in groups 2—3, 5—6, and 8—9, when compared with those in groups 1, 4, and 7, respectively.

** Recipients received neither untreated nor normal serum-treated thymocytes.

Table 3. Demonstration of TS_{artl} and TS_{gvhr} activities in the cell populations of the immune organs from TBE virus-infected mice

Group of mice	Donors	Recipients	Characteristics of donor cells[1]	Index of GVHR[2]
			TS_{artl}	
1	CBA	CBA	S_{tbe}	2.2
2	CBA	CBA	$S_{tbe} + T_{tbe}$	1.4
3	CBA	CBA	$S_{tbe} + T_{tbe}$-mit C	1.3
4	CBA	CBA	$S_{tbe} + LNC_{tbe}$	1.5
5	CBA	CBA	$S_{tbe} + LNC_{tbe}$-mit C	1.4
6	CBA	CBA	S_{tbe}-mit C	1.1
7	CBA	CBA	$S_{tbe} + S_{tbe}$-mit C	2.1
			TS_{gvhr}	
8	CBA	BALB/c	S	2.9
9	CBA	BALB/c	$S + S_{tbe}$	1.9
10	CBA	BALB/c	$S + S_{tbe}$-mit C	1.8
11	CBA	BALB/c	$S + T_{tbe}$	1.7
12	CBA	BALB/c	$S + T_{tbe}$-mit C	1.8
13	CBA	BALB/c	$S + LNC_{tbe}$	1.9
14	CBA	BALB/c	$S + LNC_{tbe}$-mit C	1.8

[1] Uninfected CBA or BALB/c recipients were inoculated into the footpad with 10^7 splenocytes from TBE virus-infected (S_{tbe}) or uninfected (S) CBA donors or with their mixture with the same amount of syngeneic thymocytes (T_{tbe}) or lymph node cells (LNC_{tbe}) from TBE virus-infected donors, either treated before with mitomycin C (mit C) in vitro or untreated.

[2] Statistically significant differences ($p \leq 0.05$) for TS_{artl} only in groups 1 and 7, for TS_{gvhr} only in the group 8.

but not by sera of Balb/c (H-2^{dd}), DBA (H-2^{dd}) and C57Bl (H-2^{bb}) mouse lines or F_1 (BALB/cxC57Bl) (H-2^{db}) hybrids (Table 1). Addition of TS_{artl} to ARTL was accompanied by a regular inhibition of GVHR induced in syngeneic recipients. Analogous results were obtained with thymocytes and splenocytes from mice infected with other flaviviruses used, namely with yellow fever, Langat and D2 viruses (results not presented). Activity of the serum factor was not connected with destruction of TS_{artl}, because the proportion of destroyed thymocytes incubated in vitro either with medium 199 in HBSS or with mouse serum was the same, not exceeding 3—5%.

As demonstrated previously, TBE virus infection of mice was accompanied by appearance of T-suppressors inhibiting the immune aggression of allogeneic lymphocytes against their recipient (Khozinsky and Semenov, 1981). These suppressors were not sensitive to the effect of pooled normal mouse serum inhibiting TS_{artl}. When adding to normal splenocytes, the TS_{gvhr} treated in vitro with serum of syngeneic donors, the suppressive effect was still preserved, but the index of GVHR induced by this mixture was markedly lower than in controls (Table 2). The demonstrated difference between TS_{artl} and TS_{gvhr} by the use of normal serum made it possible to suggest that they could belong to different subpopulations of T-lymphocytes. This suggestion is supported by a different distribution of either type of suppressors in organs of the immune system: TS_{gvhr} could be found in thymus, lymph nodes and spleen, but TS_{artl} in thymocytes and lymph nodes only.

To confirm further this suggestion, the mitomycin C-treated splenocytes, thymocytes and lymph node cells from virus-infected mice were employed as possible source of TS_{artl} for the inhibition of ARTL activity. As follows from Table 3, mitomycin C treatment did not affect activities of TS_{artl} and TS_{gvhr} present in the cell populations from thymus and lymph nodes. However, the mitomycin C-treated splenocytes displayed only TS_{gvhr} activity, but not TS $_{artl}$ activity. They did not inhibit the virus-induced ARTL activity in syngeneic mice, even if increasing the ratio of mitomycin C-treated splenocytes to ARTL 4 : 1 (data not presented).

Discussion

The question of the role of blocking factors in the pathogenesis of virus infections remains open, though the existence of such factors is undoubted. The best known are the effects of virus-induced factors of immunoglobulin nature, targets of which are the effectors of the immune response (Swick *et al.*, 1976; Veltri *et al.*, 1981). We described earlier a factor in the serum of normal mice which inhibited in vivo the activity of virus-induced ARTL. The serum factor activity was H-2 restricted: the suppressing effect developed only providing that the H-2 antigens of ARTL were completely or partially identical with H-2 antigens of normal serum donors (Khozinsky and Semenov, 1983). As follows from results of our present paper, the normal mouse serum was capable to inhibit the activity of flavivirus-induced ARTL; this phenomenon was again H-2 dependent.

Thus, our data indicate the presence of factor(s) in the normal mouse serum directed to the effectors of autoimmune response, i.e. to ARTL, as well as to the cells regulating this response, i.e. to TS_{artl}. The nature of the factor(s) and their possible difference or identity is so far unknown. The factor inhibiting TS_{artl} was not effective against TBE virus-induced T-suppressors, which were directed to the immune aggression of allogeneic lymphocytes in vivo. This fact and the finding of different distribution of TS_{artl} and TS_{gvhr} in organs of the immune system makes it possible to suggest that flavivirus infection is accompanied by appearance of different subpopulations of nonspecific T-suppressors. The relativity of the term „nonspecific" should be stressed: T-suppressors are not specific in relation to the inducing virus, but they are highly specific for ARTL and GVHR effectors.

At present the appeareance of ARTL is connected with a selection of T-lymphocytes bearing on their membrane high affinity receptors for cell-to-cell interactions, which are capable to distinguish self-antigens (Altman and Katz, 1980). Apparently in virus infection the TS_{artl} are selected in parallel with ARTL selection. It can be assumed that decrease in the level of normal serum factor(s) inhibiting both ARTL and TS_{artl} favoured ARTL and TS_{artl} selection during virus infections. This assumption should be verified, though it is supported by the model of Cohen and Wekerle (1977) showing activation of autoreactive lymphocytes in a syngeneic culture using medium devoid of the blocking serum factor.

References

Altman, A., and Katz, D. H. (1980): The induction of autoreactive T-lymphocytes by allogeneic effect factor (AEF) relevance to normal pathway of lymphocyte differentiation. *Immunol. Rev.* **51**, 3—34.

Cohen, I. R., and Wekerle, H. (1977): Autoimmunity, self-recognition and blocking factors, pp. 231—265. In N. Talal (Ed.): *Autoimumnity, Genetic, Immunologic, Virologic and Clinical Aspects.* Academic Press, New York.

Cooke, A., and Lydyard, P. M. (1981): The role of T-cell in autoimmune diseases. *Path. Res. Pract.* **171**, 173—196.

Cunningham, A. J. (1976): Self-tolerance maintained by active suppressor mechanisms. *Transpl. Rev.* **31**, 23—43.

Denman, A. M., Pelton, B. K., Appleford, D., and Kinsley, M. (1976): Virus infections of lympho--reticular cells and auto-immune disease. *Transpl. Rev.* **31**, 79—115.

Jerne, N. K. (1973): The immune system. *Sci. Amer.* **229**, 52—63.

Khozinsky, V. V., and Semenov, B. F. (1980): Nonspecific T-suppressors, eliminating the function of autoreactive cells in experimental flavivirus infections (in Russian). *Immunologiya* **1980** (5), 57—60.

Khozinsky, V. V., and Semenov, B. F. (1981): Nonspecific T-suppressors in experimental tick--borne encephalitis. *Acta virol.* **25**, 277—282.

Khozinsky, V. V., and Semenov, B. F. (1983): Serum factor blocking the activity of virus-induced autoreactive T lmphocytes of mice. *Acta virol.* **27**, 346—350.

Levy, J. A. (1974): Autoimmunity and neoplasia. The possible role of C-type viruses. *Am. J. clin. Path.* **62**, 258—280.

Semenov, B. F., Khozinsky, V. V., and Vargin, V. V. (1974): Delayed hypersensitivity in acute and latent flavivirus infections (in Russian). *Vestn. Akad. med. Nauk* **9**, 60—64.

Semenov, B. F., Kaulen, D. R., and Balandin, I. G. (1982): In E. A. Gogolina (Ed.): *Kletochnye i molekularnye Osnovy protivovirusnogo Immuniteta (in Russian).* Meditsina, Moskva, p. 239.

Swick, H. M., Brooks, W. H., Roszman, T. L., and Galdmell, D. (1976): A heat-stable blocking factor in the plasma of patients with subacute sclerosing panencephalitis. *Neurology* **26**, 84—86.

Talal, N., Roubinian, J. R., Shear, H., Horn, J. T., and Miyasaka, N. (1980): Progress in mechanisms of autoimmune disease, pp. 889—905. In M. Fougerean and J. Dausset (Eds): *Progr. Immunol.* **4**, Academic Press, New York.

Veltri, R. W., Kikta, V. A., WainWright, W. H., and Sprincle, P. M. (1981): Biological and molecular characterization of the IgG serum blocking factor (SBF-IgG) isolated from sera of patients with EBV-induced infectious mononucleosis. *J. Immunol.* **127**, 320—328.

Wekerle, H. (1978): Immunological T-cell memory in the in vitro-induced experimental autoimmune orchitis. Specificity of the reaction and tissue distribution of the autoantigens. *J. exp. Med.* **147**, 233—250.