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H-2 RESTRICTED REGULATION BY SOLUBLE AUTOANTIGENS OF THE TICK-BORNE ENCEPHALITIS VIRUS-INDUCED AUTOREACTIVE T-EFFECTOR AND T-SUPPRESSOR LYMPHOCYTES IN MICE

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Summary. — Soluble autoantigens (mouse red blood cells lysed by sonication) blocked in vitro the antigen-recognizing receptors of tick-borne encephalitis (TBE) virus-induced autoreactive T-lymphocytes (ARTL), effectors of the local graft-versus-host reaction (GVHR) in a syngeneic system and prevented the development of GVHR in vivo. Antigen-recognizing receptors were also found on T-suppressors (T_s) that became activated during experimental tick-borne encephalitis in mice and inhibited the activity of ARTL. The interaction between these receptors and autoantigens in vitro resulted in a loss of the ability of T_s to inhibit in vivo the ARTL-mediated GVHR. A similar result was obtained with ARTL and T_s activated in mice infected with Langat, dengue type 2 (D2) and yellow fever (strain 17D) viruses. The block of the antigen-recognizing receptors of T-cells was reversible, and not associated with lymphokine production or effector death. The block of the antigen-recognizing receptors in vitro and the loss of the corresponding T-cell function in vivo occurred provided that the donors of soluble erythrocyte antigens (SEA) and of the lymphocytes had at least one common major histocompatibility complex (MHC) haplotype. Injection SEA from donors whose H-2 complex haplotypes were identical to those of TBE-infected recipients prevented in the latter the formation of ARTL or T_s. The autoantigens inhibiting the ARTL and T_s activities seemed to be products of the MHC genes. The role of soluble H-2 autoantigens in preventing virus-induced autoimmune reaction and maintaining a state of natural immunological tolerance is discussed.

Key words: flaviviruses; soluble H-2 autoantigens; autoreactive T-lymphocytes; T-suppressors; autoimmunity; natural immunological tolerance

Introduction

The specificity of effector lymphocytes is based on the presence of specialized surface receptors. These receptors mediate the specific recognition and binding of various antigens (Brondz and Rokhlin, 1978). A group of extracellular substances exists (so-called blocking factors) which also may prevent differentiation and/or interaction of lymphocytes with their targets by specific binding to antigen-recognizing receptors. These factors include antigens, antibodies to these antigens within the immune complex or antiidiotypic antibodies (Cunningham, 1976; Binz and Wigzell, 1977). Numerous experiments have demonstrated the occurrence of a block of antigen-recognizing receptors by soluble antigens (including H-2) which results in inability of lymphocytes to lyse tumors or allogenic cells in culture, to induce GVHR in allogenic animals or to produce antibodies (Halle-Panenko *et al.*, 1976; Cohen and Wekerle, 1977). The blockade by autoantigens of receptors on ARTL recognizing these autoantigens may be a mechanism of maintaining the state of natural immunological tolerance. A number of workers believe that an important role in maintaining this state is played by concentration of autoantigens in the microenvironment of autoreactive cells, i.e. the balance between the states of tolerance and autoimmunity depends on the relative amount of autoreactive cells with blocked and free autoantigen-recognizing receptors (Weigle, 1980). Thus, the protective effect of basic myeline protein in experimental allergic encephalomyelitis may be explained by the blockade of autoreactive lymphocytes (Arnon, 1981). According to the hypothesis proposed by Cohen and Wekerle (1977), the presence of soluble autoantigen in animal serum ensures the state of natural immunological tolerance by blocking the antigen-recognizing receptors of autoreactive lymphocytes. Evidence suggesting the involvement of soluble H-2 antigens in preventing the recognition of autoantigens by ARTL has been reported by Sia and Parish (1980). It has been demonstrated that soluble H-2 antigens present in a lysate produced by sonication of mouse red blood cells prevented the formation of rosettes in vitro with autologous red blood cells or normal mice lymphocytes.

Earlier we found that the splenocytes of mice subjected to experimental flavivirus infections contained ARTL inducing a local GVHR in syngeneic uninfected recipients. The activity of these cells was controlled by virus--induced T_s which were activated in the populations of thymocytes and lymph node cells of infected mice (Vargin and Semenov, 1980; Khozinsky and Semenov, 1980). The ARTL and T_s activities are controlled by serum blocking factor(s) (SBF) present in the sera of normal mice of common MHC haplotypes (Khozinsky and Semenov, 1983, 1984). The activation of the both T-lymphocyte subpopulations in infected animals is correlated with the virus-induced decrease of the SBF activity. Based on these results we propose a hypothesis regarding the drop of SBF activity in viral infections for a factor predisposing to the development of autoimmune reactions (Khozinsky and Semenov, 1984*a*).

In this report we present evidence suggesting that the circulating soluble H-2 autoantigens may play a role in regulating the activity of virus-induced ARTL and Ts and in peventing the autoimmune reactions in viral infection.

Materials and Methods

Virus strains. TBE virus Sofyin and Langat strains (TP-21), dengue 2 strain No. 23085 and yellow fever strain 17D were grown in the brain of 2 to 3-day-old mice. They were inoculated intraperitoneally (i.p.) to mice in a dose of 10^4 LD_{50}/0.3 ml diluted in medium 199 prepared in Hanks' solution. The viruses were titrated in suckling mice by intracerebral (i.c.) inoculation with serial suspension dilutions.

Mice. Lines BALB/c, CBA, C57B1, DBA, AKR and their first generation hybrids F_1 (CBA × × C57B1), F_1 (CBA × BALB/c) and F_1 (DBA × C57B1) weighing 18–20 g were obtained from the Stolbovaya nursery of the U.S.S.R. Academy of Medical Sciences. In each series of experiments mice of the same sex were used.

The methods of isolation of splenocytes and thymocytes were described previously (Khozinsky and Semenov, 1983).

Autoantigens were obtained by sonication of a suspension of mouse red blood cells as described by Sia and Parish (1980). An erythrocyte pellet was sonicated with an UZDN-2T ultrasonic generator for 10–20 sec (at 30 W). The obtained transparent fluid was used for treating the immunocompetent cells. Mouse sera were prepared as described earlier (Khozinsky and Semenov, 1983). Sera without signs of haemolysis were used throughout .

ARTL and T_s were either splenocytes or thymocytes, from mice infected with one of the viruses in question, obtained on day 7 post-infection (p.i.). Uninfected recipients were given into hind footpad one subcutaneous (s.c.) injection of 10^7 splenocytes from infected syngeneic donors (ARTL) suspended in 0.1 ml of medium 199 with the Hanks' solution, or a mixture of these cells with an equal quantity of syngeneic thymocytes coming from infected donors (T_s). The GVHR index was calculated as a mass ratio of isolated colateral lymph nodes from the inoculated and control foot 6 days after injection. The presence of suppressors was indicated by a relatively lower GVHR intensity in case of administration of ARTL + Ts in comparison to ARTL alone (Khozinsky and Semenov, 1980).

The effects of SEA or SBF on ARTL or T_s activities were studied in vitro. ARTL or Ts were treated for 1 hr at 4 °C with erythrocyte lysate or control serum from normal donors at a ratio of 10^7 cells per 0.1 ml. After treatment, the cells were twice washed with 5 ml of medium 199 prepared in Hanks' solution.

SEA were abolished by 3 times repeated absorption of splenocytes or thymocytes from intact or infected donors for 1 hr at 4 °C to mouse red blood cell lysate at a ratio of 5×10^7 cells to 0.1 ml of SEA. The ARTL or T_s were devoid of SEA by means of 8 successive washings of 5×10^7 cells in fresh 5 ml portions of medium 199 in Hanks' solution, followed by centrifugation at 1000 rev/min for 10 min.

In some experiments the sonicated red blood cells diluted 1 : 3 with medium 199 in Hanks' solution were injected intravenously to recipients in 0.3 ml vol daily for 4 days.

The results were statistically treated using the Student's criterion.

Results

As is shown in Table 1, TBE virus-induced ARTL lost their ability to recognize antigens of syngeneic mice as genetically foreign after pretreatment with SEA in vitro only when the T-cell and the red blood cell donors had common H-2 gene complex haplotypes. Thus, the ARTL of BALB/c mice ($H\text{-}2^{dd}$) failed to proliferate in popliteal lymph nodes of syngeneic recipients after contact with SEA of their syngeneic donors DBA ($H\text{-}2^{dd}$), hybrids F_1 (CBA × BALB/c) ($H\text{-}2^{kd}$) and F_1 (DBA × C57B1) ($H\text{-}2^{db}$) but not of CBA ($H\text{-}2^{kk}$), AKP ($H\text{-}2^{kk}$) or C57B1 ($H\text{-}2^{bb}$) or hybrids F_1 (CBA × C57B1)

Table 1. Demonstration of the H-2 restriction of inhibitory effects of SEA on the virus-induced ARTL and T_s activites

Group	Origin of SEA	H-2 haplotype	GVHR index					
			ARTL from mouse lines			ARTL + T_s from mouse lines		
			BALB/c	CBA	C57Bl	BALB/c	CBA	C57Bl
1	—**	—	2.1	2.2	2.1	1.3	1.4	1.3
2	CBA	H-2^{kk}	2.0	1.1*	2.0	1.4	2.0*	1.3
3	C57Bl	H-2^{bb}	1.9	2.1	1.1*	1.3	1.3	2.0*
4	BALB/c	H-2^{dd}	1.2*	2.3	2.1	2.2*	1.3	1.3
5	DBA	H-2^{dd}	1.2*	2.1	2.0	2.1*	1.3	1.3
6	AKR	H-2^{kk}	2.0	1.3*	2.2	1.3	2.1*	1.2
7	(CBA × C57Bl) F_1	H-2^{kb}	2.2	1.2*	1.3*	1.4	2.0*	2.2*
8	(CBA × BALB/c F_1	H-2^{kd}	1.2*	1.1*	2.0	2.3*	2.1*	1.2
9	(DBA × C57Bl) F_1	H-2^{db}	1.2*	2.0	1.2*	2.1*	1.3	2.2*

Notice: The source of ARTL were splenocytes obtained from mice i.p. infected with TBE virus (10,000 LD_{50}/0.3 ml into the abdominal cavity) on day 7 post-infection. 1×10^7 splenocytes (intact or treated with SEA of a studied mouse line) were injected by s.c. route into the right foot of noninfected syngeneic mice. The source for T_s were splenocytes of the same infected mice. Intact or SEA-treated thymocytes were added to intact syngeneic ARTL. The mixture was inoculated subcutaneously into the right foot.

* Inhibition of ARTL or T_s activity; the values were significantly different from those of other groups ($P \leq 0.005$).

**ARTL or T_s were not treated with SEA.

(H-2^{kb}) (Table 1, groups 1 and 4, 5, 8, 9; 1 and 2, 3, 6, 7). In alternative experiments, the ability of ARTL from CBA mice (H-2^{kk}) to induce a local GVHR in syngeneic recipients was suppressed only after contact with the SEA of donors having at least one allele (k) of the H-2 gene complex in common with T-cell donors (Table 1, groups 1 and 2, 6, 7, 8). The absence of this allele in red blood cell donors C57Bl (H-2^{bb}), BALB/c (H-2^{dd}), DBA (H-2^{dd}) or hybrids F_1 (DBA × C57Bl) (H-2^{db}) abolished the ARTL activity inhibition phenomenon (Table 1, group 1 and 3, 4, 5, 9). Correspondingly, the ARTL of line C57Bl (H-2^{bb}) treated with SEA of syngeneic donors or of F_1 (CBA × × C57Bl) hybrids (H-2^{kb}) or F_1 (DBA × C57B17) hybrids, but not with SEA of CBA (H-2^{kk}), AKR (H-2^{kk}), BALB/c (H-2^{dd}), DBA (H-2^{dd}) or F_1 (CBA × BALB/c) (H-2^{kd}) mice, were unable to induce GVHR in syngeneic recipients (Table 1, groups 1 and 3, 7, 9; 1 and 2, 4, 5, 6, 8).

To study the effect of SEA on T_s activity, the latter were treated in vitro with SEA, then mixed with intact ARTL and injected into the syngeneic recipients. As evident from Table 1, the T_s of BALB/c mice lost their ability to inhibit ARTL-induced local GVHR after pretreatment with SEA of syngeneic donors DBA (H-2^{dd}) or F_1 (CBA × BALB/c) (H-2^{kd}) and F_1 (DBA × C57Bl) (H-2^{db}) hybrids which had common H-2 haplotypes with the T_s donors (Table 1, groups 1 and 4, 5, 8, 9). On the other hand, SEA

prepared from red blood cells of CBA (H-2^{kk}), AKR (H-2^{kb}), C57Bl (H-2^{bb}) or F_1 (CBA × C57Bl) (H-2^{kb}) mice which had no common MHC haplotypes with the BALB/c T-cells failed to affect the activity of immunoregulating cells (Table 1, groups 1 and 2, 3, 6, 7). In alternative experiments with T_s derived from CBA or C57Bl mice there was also a clear relationship between the ability of SEA to suppress the T_s activity and the existence of common H-2 haplotypes in the donors of red blood cells and T-cells (Table 1, groups 1 and 2, 6, 7, 8; 1 and 3, 4, 5, 9; 1 and 3, 7, 9; 1 and 2, 4, 5, 6, 8).

Similar results with regard to interaction between SEA and ARTL or Ts in different H-2 backgrounds were obtained with T-cells of BALB/c mice infected with the Langat, D2 or yellow fever (strain 17D) viruses (data not shown).

In a further series of experiments with TBE virus-activated ARTL and Ts we have shown that the activity of the T-effectors is inhibited by SEA prepared from red blood cells of the same animals. In order to demonstrate

Table 2. The reversible, H-2-restricted adsorption of SEA on the surface of ARTL and T_s which control ARTL induced in mice by TBE virus

Group	Treatment of the T-cells: Intact SEA from CBA mice	SEA exhausted on mouse immunocompetent cells: intact	pretreated: no washing	pretreated: washing before exhaustion	washing before injection to recipients	GVHR index: ARTL	GVHR index: ARTL + T_s
1	–	–	–	–	–	2.2	1.3
2	+	–	–	–	–	1.2*	2.1*
3	+	–	–	–	+	1.9	1.4
4	–	–	CBA	–	–	1.3*	2.0*
5	–	–	BALB/c	–	–	2.1	1.3
6	–	–	CBA	+	–	2.0	1.4
7	–	CBA	–	–	–	2.1	1.3
8	–	AKR	–	–	–	2.0	1.2
9	–	(CBA × C57Bl) F_1	–	–	–	2.2	1.4
10	–	BALB/c	–	–	–	1.2	2.1
11	–	C57Bl	–	–	–	1.3*	2.0*

Notice: In studies on ARTL and T_s, respectively, the splenocytes and thymocytes of infected CBA mice were treated with SEA of syngeneic donors or similar SEA preadsorbed on splenocytes (in case of ARTL) or on thymocytes (in case of T_s) taken from different infected mouse lines. The splenocytes and thymocytes were obtained on day 7 after i.p. infection of mice with 10,000 LD_{50}/0.3 ml of TBE virus. For antigen elution the ARTL or T_s were washed 8 times with medium 199 in Hanks' solution. In the case of T_s investigation, an equal amount of intact or appropriately treated thymocytes from the same donors was added to intact splenocytes of infected mice. (+) or (–) denote that the corresponding procedure was or was not performed.
For further explanations see Legend to Table 1.

this, we took blood samples from some animals twice at an interval of 24- or 48-hr from the retroorbital sinus and stored the blood in an Alsever's solution. The blood donors were then infected with the TBE virus, and 96 hr later ARTL and T_s were obtained separately from each animal. The cell samples were then individually treated with SEA obtained from the same animal (data not shown). In no cases did the treatment of splenocytes or thymocytes of infected donors with SEA not result in death of immunocompetent cells. The fraction of dead cells in the treated and control samples was the same and equal to 5% under the conditions used.

The data summarized in Table 2 indicate that the suppression of ARTL or T_s activities after treatment with SEA obtained from syngeneic animals was due to sorption of an inhibiting substance on the immunocompetent cells. This conclusion is based on the following facts: 1. The soluble erythrocyte antigens lose their ability to inhibit ARTL and T_s activities after a 3-fold adsorption to splenocytes and thymocytes from TBE-infected mice (Table 2, groups 1 and 7). 2. The loss of the inhibiting activity of SEA was accompanied by a drop of ARTL and T_s activities in the populations of spleen and/or thymic cells, respectively, used for the adsorption (data not shown). 3. ARTL or T_s treated with SEA in vitro restored their functional activity in vivo after many washes with fresh medium (see Table 2, groups 1 and 3). 4. ARTL and T_s pretreated with SEA from syngeneic but not completely H-2 identical animals were unable to abolish the SEA studied, but numerous washings restored the ability of ARTL to induce GVHR and of T_s to inhibit ARTL activity and exhaust SEA (Table 2, groups 1 and 4, 5; 1 and 6). Reversible sorption of the inhibiting substance of SEA onto the studied T-cell populations is of specific nature determined by the presence of at least a single common H-2 haplotype in the donors of red blood cells and of ARTL or T_s. This fact was established in experiments in which SEA of DBA mice were exhausted with populations of ARTL- or T_s-containing immunocompetent cells taken from donors with different MHC haplotypes. The SEA became unable to inhibit the ARTL-induced GVHR in CBA mice ($H\text{-}2^{kk}$) if the exhaustion treatment was done with ARTL of syngeneic AKR mice ($H\text{-}2^{kk}$) or F_1 (CBA $\times$ C57B1) hybrids ($H\text{-}2^{kb}$) but not of BALB/c ($H\text{-}2^{dd}$) or C57B1 ($H\text{-}2^{bb}$) mice (Table 2, groups 1 and 7, 8, 9; 1 and 10, 11). Similar results were obtained with SEA pools adsorbed with thymocytes of TBE virus-infected mice. SEA prepared from red blood cells of CBA ($H\text{-}2^{kk}$)mice lost the ability to inhibit the functional activity of T_s from syngeneic mice after absorption to thymocytes from infected CBA, AKR or F_1 (DBA $\times$ C57B1) donors, but it remained inhibitory to Ts after adsorption with thymocytes of infected BALB/c or C57B1 mice lacking any common haplotypes with CBA mice (Table 1, groups 1 and 7, 8, 9; 1 and 10, 11). In all experiments, the splenocytes and thymocytes from uninfected donors did not exhaust SEA under similar experimental conditions (data not shown).

SEA were capable of inhibiting the activity of virus-induced ARTL and Ts not only after treating the effectors in vitro but also directly in vivo. This is implied by experiments in which SEA of syngeneic animals was

intravenously injected to CBA mice simultaneously with or on days 1, 2 and 3 p.i. with TBE virus. No ARTL or T_s were found in these mice on day 4 p.i. This was not due to in vivo suppression of the virus replication by SEA. Titres of the virus in the brain of SEA-treated and control mice were similar. The blockade of T-cell activity by SEA in vivo also followed the role of H-2 restriction. Active ARTL or T_s were not found on day 4 in CBA ($H\text{-}2^{kk}$) mice that received 4 injections (at 24 hr intervals) of SEA from AKR ($H\text{-}2^{kk}$) or F_1 (CBA × C57B1) ($H\text{-}2^{kb}$) mice, but were regularly detected in the recipients (data not shown) inoculated with SEA of BALB/c ($H\text{-}2^{dd}$) or C57B1 ($H\text{-}2^{bb}$).

Discussion

The above evidence as well as the results of our previous communication (Khozinsky and Semenov, 1984) indicate that reversible binding of an active substance to the surface structures of immunocompetent cells is indispensable for blocking ARTL and T_s activities by SEA or SBS components. Since we did not use pure subpopulations of cells, it remains not entirely clear which cells are inhibited and which are the suppressors. In theory, 3 mechanisms do account for the inhibiting action: 1. Direct block of lymphocyte activity by means of binding to an antigen-recognizing receptor. 2. The binding of an inhibitor to receptors on ARTL and T_s may be accompanied by production of a mediator, which exerts the suppressing effect. 3 The inhibitor binds to surface structures of cell populations other than ARTL or T_s, and these populations induce the observed suppression either directly or by means of a mediator. Results of experiments in which we tested the above hypothetical possibilities favour to the first. Splenocytes from infected mice, treated with SEA or with normal serum from syngeneic donors, did not affect the intensity of GVHR when added to intact ARTL, while T_s were as effective in inhibiting GVHR in the presence of treated thymocytes from infected mice as in their absence (data not shown). If the 2nd or the 3rd mechanisms were correct, addition of SEA- or serum-treated splenocytes and thymocytes to intact ARTL and T_s, would reduce the activity of these cells, which was not the case. In our opinion, therefore, the block of ARTL or T_s activities by SEA or SBF resulted from a direct binding to the T-lymphocyte surface structures. Of course, we do not rule out the possibility that the suppressive substance binds to other subpopulations of immunocompetent cells as well, but, as our experiments have shown, such a binding if exists, does not determine the suppression of the ARTL and T_s activities.

The last decade witnessed an intensive investigation of the role played by MHC genes in viral infections. The MHC genes are involved in the regulation of the cellular and humoral immunities, and in a number of cases determine the genetic resistance or susceptibility to the viral infection. Antigens encoded by various MHC subloci, which are expressed on the surface membranes of immunocytes, participate in cooperative interactions and differentiation of immunocompetent cells, in recognition of virus-induced antigens by T-killer cells on the surface membrane of target cells. MHC antigens may

serve as receptors for viruses and the latter, in turn, may either modulate the expression of MHC antigens on cellular surfaces or induce the expression of alloantigens (Sasazuki and McDevitt, 1977; Miller, 1978; Zinkernagel and Doherty, 1979; Semenov *et al.*, 1982). The majority of the above-mentioned MHC antigen functions is associated with the MHC products expressed antigens on cellular surface membranes. The part played by soluble (circulating) encoded by MHC genes in the course of viral pathology remains largely unknown, although the occurrence of such antigens in the blood and other biological fluids has been documented (Reisfeld *et al.*, 1976; Snell *et al.*, 1979).

The present report is the first demonstration that employing SEA the soluble autoantigens may regulate the activities of virus-induced ARTL and T_s inhibiting the ARTL. Earlier Sia and Parish (1980) demonstrated that SEA contained H-2 antigens preventing rosette formation in vitro of T- and B-lymphocytes from normal mice with red blood cells of donors identical in the $H\text{-}2^k$ or $H\text{-}2^d$ subloci. In our experiments an in vitro contact of SEA prepared from red blood cells of mice before their infection with ARTL or T_s induced by TBE virus in the same animals, resulted in a loss of the ARTL ability to induce local GVHR in syngeneic recipients and in a loss of T_s activity inhibiting ARTL in vivo. In order to understand the mechanism of this phenomenon we studied different combinations of red blood cell and T-cell donors having different H-2 haplotypes. It was found that suppression of the ARTL and T_s activities occurred only when the SEA were obtained from animals that were completely or partially identical to the effector donors in their H-2 haplotypes. The interaction of the immunocompetent cells with SEA was accompanied by bindig of the antigen to cellular surface structures which became unable to adsorb another antigen, while the adsorbed (exhausted) SEA were rendered unefficient to inhibit the activity of completely or partially MHC-identical ARTL or T_s. This binding of SEA, which resulted in a loss of ARTL ability to induce GVHR and of T_s ability to inhibit ARTL, and which was specific since it depended on the existence of common H-2 haplotypes in the donors of erythrocytes and T-effectors, was unstable and did not cause the death of the lymphocytes. After washing of the SEA-blocked cells their activity and ability to bind antigen was restored. The drop of ARTL or T_s activities following a contact of the cell populations with SEA or the earlier described SBF did not entail the appearance of lymphokines or an activation of cells inhibiting the effector activity.

Experiments with ARTL and T_s induced in mice by the Langat, D2 or yellow fever (strain 17D) viruses suggest that a similar mechanism of the studied T-cell blockade in the context of H-2 molecules is possible in these infections as well.

On the whole, the present results and the available evidence on the nature of T-cell interaction with blocking antigens allows the conclusion that the recognition by virus-induced ARTL of autoantigens is accomplished by receptors that recognize H-2 autoantigens (Cohen and Wekerle, 1977). In the soluble form, these antigens block receptors of ARTL inhibiting the

capability of ARTL to recognize autoantigens on cellular membranes and proliferate in response to a specific antigenic stimulus. Receptors capable of recognizing autoantigens are also present in virus-induced T_s. A blockade of these receptors by soluble H-2 autoantigens also entails a loss of activity. It should be noted that the receptors of virus-induced effectors are different from the receptors found at the surface of T-lymphocytes from normal mice. As reported by Sia and Parish (1980), these receptors failed to distinguish k and d haplotypes. In our experiments the T-cells clearly differentiated the antigens of donors with k, d or B haplotypes, which may indicate that different subpopulations of autoreactive T-lympocytes may exist being capable to recognize different antigens encoded by different subloci of the H-2 complex.

A comparison of the results of the present and previous reports suggests that the soluble H-2 autoantigen is functionally identical with the SBF described earlier (Khozinsky and Semenov, 1985). It appears that, regardless of whether the identity of the two blocking factors will be proved or refused, the presence of MHC gene products in the circulation is an indication of their possible involvement in regulation of the activity of virus-induced T-effectors of the autoimmune response as well as in the action of immunoregulating cells controlling the activity of T-effectors. A support to this assumption comes from our present finding that an injection of SEA to TBE virus-infected mice results in an H-2 restricted blockade of ARTL and T_s in vivo. It is likely that the circulating H-2 autoantigens do not only block the T-cell activity but also allow a negative selection (elimination) of lymphocyte clones with high-avidity receptors for the autoantigens at the level of precursor cells or at other earlier stages of differentiation.

In addition to stressing the role of soluble H-2 autoantigens in preventing virus-induced autoimmune reactions, the presented evidence argues for the involvement of these antigens in maintaining a state of natural immunotolerance. It also suggests that the blockade of antigen-recognizing receptors on autoreactive cells by soluble autoantigens is not the only mechanism of maintaining this state. It appears that regulation by autoantigens of the T_s control of ARTL activity represents an important factor contributing to internal immunological homeostasis.

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