SEPARATION OF ANTISUPPRESSOR RAT SERUM ANTIBODIES ELIMINATING T SUPPRESSOR CELLS AND STIMULATING THEIR FORMATION IN MICE IN VIVO

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In view of the exceptionally important role of T suppressor cells in regulation of the immune response and in the pathogenesis of many diseases, selective elimination of T suppressor cells in vivo or, conversely, artificial intensification of their activity, lead to an oriented change in the immune response, which may be important for the prevention and treatment of autoimmune and neoplastic diseases. In particular, injection of alloantibodies against one of the markers of specific T suppressor cells (STS), namely the I-J antigen of the H-2 complex, stimulates antibody formation [14], abolishes genetic areactivity to the antigen [15], and stimulates antitumor immunity [10]. This last phenomenon is particularly effective when monoclonal antibodies against extract of antitumor STS are injected into mice [13]. It is evident that the use of xenogeneic antisera against markers of T suppressor cells (or their precursors), expressed on thymoma cells [7], immature thymocytes [11], or T cells tolerant to Vi-antigen [6] or to hapten [8] is more universal. In these cases, antisuppressor sera (ASS) had an effect on the function of those categories of T suppressor cells which either suppress antibody formation of stimulated tumor growth without immunologic specificity.

An ASS which, in vitro, selectively activated STS inhibiting the proliferative response of other T lymphocytes to alloantigens in mixed lymphocytes culture (MLC), but had no effect on function of T killer cells and prodicers of macrophage migration inhibition factor (MMIF), also specific for alloantigens, was obtained by immunizing rats with enriched mouse STS. Injection of this ASS into mice prevented STS generation in vivo and inhibited growth of a syngeneic sarcoma [1].

The aim of the present investigation was to discover the effects of ASS when injected into mice before and after immunization, and also into immunized animals.

EXPERIMENTAL METHOD

BW 5147 and PDM4 thymomas were maintained in the ascites form by passage through AKR ($H\text{-}2^k$) mice, and thymoma EL4 was maintained in C57BL/6 (B6 ($H\text{-}2^b$) mice. BALB/c anti-B6 and CBA anti-B6 STS were induced by intravenous injection of $9 \cdot 10^7$ B6 spleen cells, irradiated in a dose of 2000 rads. After 4 days the spleen cells were treated with mitomycin C, added to a one-way MLC of the same specificity in the ratio of 1:3 to reacting lymphocytes, and the index of inhibition (II) of DNA synthesis was determined [5].

To obtain ASS Wister rats were immunized with BALB/c anti-B6 STS [1], enriched by adsorption-elution on a monolayer of macrophages [4], and the resulting antiserum was exhausted by erythrocytes and mouse lymph node cells [1]. Anti-I-J^k serum was obtained by immunizing B10.A(3R) mice with B10.A(5R) spleen cells [3]. Serum antibodies against I-J^k and Thy-1.2 (from Searle Diagnostics, England) and monoclonal antibodies against Lyt-antigens were tested in the microcytotoxic reaction, using rabbit complement (from Cedarlane, Canada). Titers of antisera against Thy-1.2 in the reaction with CBA mouse thymocytes and against I-J^k in the reaction with eluted B10.A(2R) anti-B10 STS were 1:64 and 1:16 respectively. Monoclonal anti-Lyt-antibodies were discovered in titers of 1:6400 for anti-Lyt-2.1 and over 1:25,000 for anti-Lyt-1.1 in the reaction with CBA thymocytes.

To inactivate the STS in vitro 10^5 cells were incubated with 50 μl of antibodies for 30 min at 20°C, then with 50 μl of complement for 60 min at 37°C. ASS or normal rabbit serum (NRS), in a dose of 50 μl, was in-

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TABLE 1. Inactivation of CBA Anti-B6 STS by Antibodies in Presence of Complement in Vitro

Antibodies for treating STS	II of DNA synthesis in MLC (M±m, data of 4-8 experiments)
Without treatment	37,7±2,5
NRS (1 : 4)	39,1±2,1
ASS (1 : 4)	4,1±0,8
NMS (1 : 10)	52,1±8,3
Anti-Thy-1.2 (1 : 20)	9,7±2,1
Anti-Lyt-2.1 (1 : 500)	12,9±3,6
Anti-Lyt-2.1 (1 : 500)	12,9±3,6
Anti-$I\text{-}J^k$ (1 : 4)	24,9 (21,7, 28,2)*

Legend. *) Results of two experiments. NMS) Normal mouse serum.

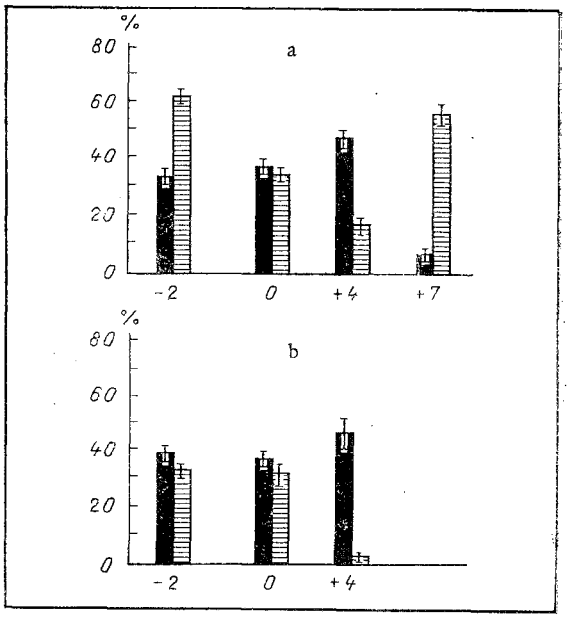

Fig. 1. Effect of ASS on T suppressor cell generation in vivo. Ordinate, II of DNA synthesis on addition of spleen cells of BALB/c or CBA mice, receiving an injection of NRS (black columns) or ASS (horizontally shaded columns) 2 days before immunization (−2), simultaneously with immunization (0), or 4 days after immunization (+4), or without immunization (+7). a) Injections of sera without complement, b) with complement. Each column − M±m for results of 3-5 experiments.

jected intraperitoneally into mice in a single dose of 0.2 ml together with 100 μl of complement at various times relative to immunization, or without complement and without immunization. In the last case, STS activity was determined 7 days after injection of ASS.

To adsorb the antibodies 0.2 ml of antiserum was incubated with $2 \cdot 10^7$ cells for 1 h at 4°C. The splenic T cells were purified by removal of B cells on a plate covered with pure antibodies against immunoglobulin [12], and the splenic B cells were purified by removal of T cells, killed with anti-Thy-1.2 and complement, and sedimented on Ficoll-Hypague with a density of 1.09 [9]. Medullary thymocytes were obtained 2 days after injection of 2.5 mg hydrocortisone into a mouse. After preliminary treatment of the cells with antibodies $2 \cdot 10^7$ CBA thymocytes or 10^7 CBA anti-B6 immune T cells were incubated for 30 min at 20°C with 0.3 ml and 0.15 ml respectively of the preparation, washed thoroughly, and used to adsorb the antiserum in the same volume.

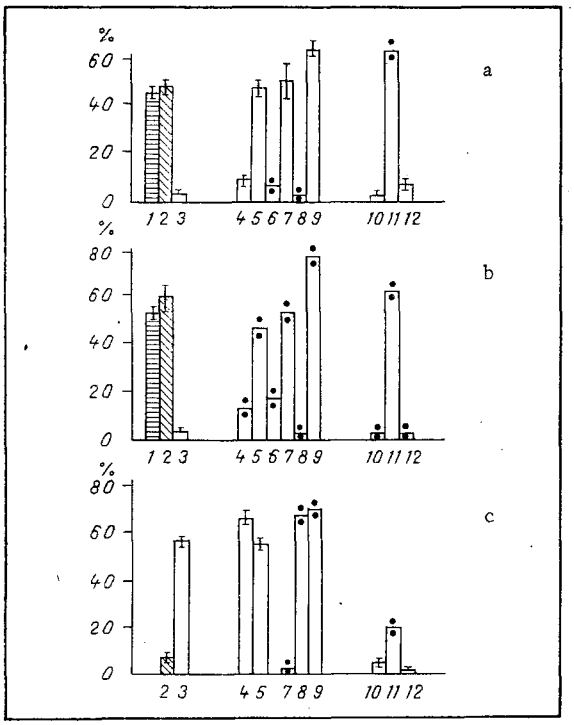

Fig. 2. Abolition of effects of ASS by adsorption with cells of different origin. Ordinate, II of DNA synthesis in MLC on addition of BALB/c anti-B6 suppressors inactivated in vitro (a), or in vivo, by injection of ASS with complement 4 days after immunization (b), or induced in vivo by ASS without immunization (c). 1) Suppressors untreated, 2-12) suppressors treated with NRS, ASS, ASS adsorbed with normal T cells, with immune T cells, with immune B cells, thymocytes, and medullary thymocytes enriched with STS, and cells of thymomas EL4, RDM4, and BW5147. Each column shows M±m for data of 3-11 experiments. Dots show results of single experiments.

EXPERIMENTAL RESULTS

Rat ASS, like antibodies against Thy-1, Lyt-2, and I-J, inhibit STS function in vitro; the inactivating effect of ASS, moreover, was the strongest (Table 1). These results, in agreement with our previous data [2, 3], characterize the phenotype of STS immune to H-2 antigens: Thy-1^+, Lyt-2^+, I-J^+.

It will be clear from Fig. 1 that injection of ASS into the mice 4 days after immunization (2 h before sacrifice) reduced STS activity, which disappeared completely if ASS was injected together with complement. Conversely, injection of ASS before immunization, or even into nonimmunized mice, led to marked activation of suppressor cells in the spleen. In both cases, on injection of allogeneic cells, or without them, suppressors induced by ASS were nonspecific, for they inhibited to a similar degree DNA synthesis in MLC, induced by stimulators of different origin: not only B6 (H-2^b, MLs^b), but also DBA/2 (H-2^d, MLc^d) (MLs denotes minor locus of lymphocyte stimulation).

To study whether the three effects (inactivation of STS in vitro and in vivo and stimulation of their formation in vivo) are due to the action of ASS, the serum was adsorbed with cells of different origin.

It will be clear from Fig. 2 that one effect of ASS is not abolished by normal T lymphocytes and medulary thymocytes. The first two effects of ASS likewise were not abolished by exhaustion with immune B lymphocytes. Conversely, all three effects were completely abolished by exhaustion with total thymocyte population, and also with thymoma RDM4 cells. The most important fact is that both inactivating effects of ASS (in vitro

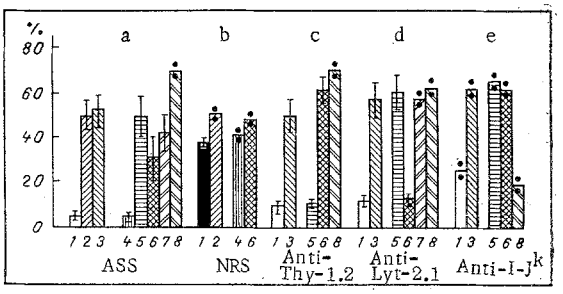

Fig. 3. Exhaustion of antibodies inactivating STS by thymocytes and immune T cells covered with antibodies. Ordinate, II of DNA synthesis in MLC during treatment of STS with anti-B6 ASS 1 : 4 (a), with NRS 1 : 4 (b), anti-Thy-1.2 1 : 20 (c), anti-Lyt-2.1 1 : 500 (d), and anti-I-J^k 1 : 4 (e). 1) Intact sera (or antibodies); 2) sera adsorbed with intact cells, 3a) sera adsorbed with cells treated with NRS (1 : 4); 3c-e) sera adsorbed with cells treated with NMS (1 : 10); 4) ASS (1 : 4); 5) anti-Lyt-1.2 (1 : 10); 6) anti-Lyt-2.1 (1 : 100); 7) anti-Lyt-1.2 (1 : 100); 8) anti-I-J^k (1 : 4). Each column represents M±m for 4-8 experiments. Dots indicate results of single experiments.

and in vivo), on the one hand, and the stimulating effect of ASS on regeneration of STS in vivo, on the other hand, can be abolished by adsorption with different cells. In particular, the first two effects disappeared after adsorption with immune T cells and with enriched STS, but not with thymus EL4 and BW5147. On the other hand, the stimulating effect of ASS on STS was unchanged after exhaustion of the ASS with immune T cells and enriched STS, but was neutralized by cells of thymomas EL4 and BW5147 (Fig. 2). ASS can evidently be divided into two types of antibodies, one of which reacts with the marker of effector STS, whereas the other type does not react with this marker, but reacts with thymoma cells, including thymomas (EL4 and BW5147) which do not carry the marker of effector STS. Inactivation of effector suppressors by ASS and stimulation of their formation in vivo are thus brought about by antibodies to different markers. Since both these markers are expressed in the thymus, but not on medullary thymocytes or normal splenic T cells, this suggests that they are represented in the lymphoid tissue of normal animals only on immature thymocytes.

Since STS are inactivated in vitro not only by ASS, but also by antibodies against Thy-1, Lyt-2, and I-J (Table 1), to study the connection between the effect of ASS and the above-mentioned antibodies ASS was exhausted with cells covered with these antibodies. It will be clear from Fig. 3 that the inactivating effect of ASS on STS was abolished by adsorption of ASS with intact or NRS-treated thymocytes or with immune T cells. The same cells, treated with ASS, lost their ability to exhaust ASS. Conversely, this ability was preserved after preliminary treatment of the cells with anti-Thy-1.2, anti-Lyt-1.1, and anti-I-J^k antibodies, whereas treatment with anti-Lyt-2.1 antibodies reduced the ability of the cells to adsorb ASS partially. It is evident that the effect of ASS is not connected with antibodies against Thy-1 or I-J, but is partly overlapped by antibodies against Lyt-2. The last effect may be due to the close mutual arrangement of antigens on the membrane reacting with ASS and anti-Lyt-2.

The results given in Fig. 3 may be associated with technical defects: shedding of "preliminary" antibodies from the surface of the cells during subsequent absorption of ASS or incomplete screening by "preliminary" antibodies of the corresponding determinants on the cell surface. Both these possibilities are unlikely: First, incubation of NRS with cells treated with ASS or anti-Lyt-2 does not lead to the appearance of antisuppressor activity in NRS (Fig. 3b), which could be caused by antibodies shed into NRS, and second, the ability of antibodies against Thy-1.2, Lyt-2.1, and I-J to inactivate STS is not reduced after their adsorption with cells treated beforehand with the corresponding antibodies: Thy-1.2, Lyt-2.1, and I-J, but it is completely abolished if the adsorbing cells are treated with NMS or with "foreign" antibodies (Fig. 3). It follows from these data that preliminary treatment with antibodies under the experimental conditions used leads to complete and stable screening of determinants on the cell surface.

Thus elimination of suppressors and their activation in vivo are induced by different antibodies of ASS, although both the corresponding antigens are expressed on cortical thymocytes. Antibodies of ASS inactivating STS do not interact under these circumstances with known STS markers – Thy-1 and I-J^k, although their partial interaction with Lyt-2 cannot be ruled out. It can be tentatively suggested that the second antibodies of ASS stimulate suppressor formation in vivo as a result of contacts with the marker of their precursors, which is absent in the effectors of STS. Such a possibility is indicated by two facts (Fig. 1): abolition of the stimulating effect of ASS by injection of complement (possibly on account of death of the precursors) and the appearance of suppressors after injection of ASS without immunization.

Isolation of two types of antibodies from ASS causing effects of elimination and activation of T suppressor cells can be used to study precursors of suppressors, to isolate purified markers of STS and their precursors, and also for deliberate modification of the immune response by abolishing or strengthening activity of suppressors in vivo.

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