by the methods used. The group of these PBG-negative tumors accounted for about 16% of the total number of cases observed.

Besides acid phosphatase, one other organ-specific antigen (PBG) was thus constantly detected in definitive, unchanged prostatic tissues. Synthesis of this antigen by cells of the principal glands commences at puberty, and is accompanied by development and considerable proliferation of the glandular tissue of the prostate. PBG is one of the secreted proteins and is constantly present in prostatic fluid and sperm.

During hyperplasia and carcinoma of the prostate the PBG level falls significantly, probably due to a decrease in the volume of normally functioning prostatic glandular tissue. The results suggest that PBG can serve as an immunochemical marker of tissue differentiation and of functional maturity of the prostate.

The immunochemical test for PBG may also find a place in the biopsy diagnosis of malignant transformation of prostatic tissue.

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PROPERTIES OF ANTIGEN-SPECIFIC SUPPRESSOR FACTOR OF IMMUNE SPLEEN CELLS

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An important role in the regulation of the immune response is nowadays ascribed to T suppressor cells (T_S), the function of which is mediated by both specific [3, 5, 11, 12] and nonspecific [4, 10, 14] factors. It was shown previously [1, 15] that after immunization of mice with sheep's red blood cells (SRBC), T_S capable of specifically suppressing the immune response of intact syngeneic recipients appeared in the animals' spleen. The supernatant (SN) obtained after ultrasonic destruction of immune spleen cells (ISC) followed by ultracentrifugation suppressed the immune response of intact recipients to SRBC [2].

The object of this investigation was to study some properties of the suppressor factor (factors) of the spleen cells of immune mice.

EXPERIMENTAL METHOD

Experiments were carried out on adult male CBA/Lac and C57BL/6 mice weighing 18-25 g, obtained from the Stolbovaya Nursery, Academy of Medical Sciences of the USSR. ISC were obtained on the 14th day after intraperitoneal injection of SRBC into mice in a dose of $5 \cdot 10^8$.

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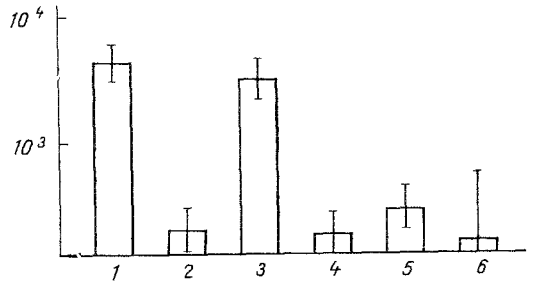

Fig. 1

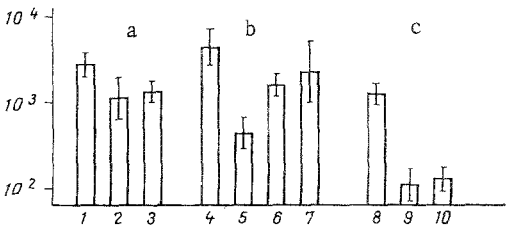

Fig. 2

Fig. 1. Effect of absorption by red blood cells on activity of suppressor factor of ISC. Here and in Figs. 2 and 3: ordinate, number of AFC in spleen of experimental CBA·Lac mice on 5th day after transplantation of NSC in a dose of $2 \cdot 10^7$ and SRBC in a dose of $2 \cdot 10^8$ cells. Confidence intervals shown at $P < 0.05$. Volume of SN added (0.5 ml) corresponds to $1 \cdot 10^8$ destroyed ISC. Conditions of absorption described in text. Donors of RRBC were August rats, of MRBC (Cab/Lac × C57BL/6)F_1 mice, and of HRBC blood group B donors, respectively. 1) NSC; 2) NSC + SN; 3) NSC + SN, absorbed with SRBC; 4) NSC + SN, absorbed with HRBC; 5) NSC + SN, absorbed with MRBC.

Fig. 2. Effect of absorption with syngeneic and allogeneic cells on suppressor activity of factor from ISC (SN) and IS of donors. NSC from C57BL/6 mice used as allogeneic cells. IS was obtained on 14th day after immunization of CBA/Lac mice with SRBC in a dose of $5 \cdot 10^8$. The dose of IS was 0.05 ml per mouse. IS was absorbed in a dilution of 1:10. a) Absorption of SN by liver cells of CBA × Lac mice: 1) NSC, 2) NSC + SN, 3) NSC + SN absorbed with liver cells. b) Absorption of SN with spleen cells: 4) NSC, 5) NSC + SN, 6) NSC + SN, absorbed with spleen cells of CBA/Lac mice, 7) NSC + SN, absorbed with spleen cells of C57BL/6 mice. c) Absorption of IS with syngeneic spleen cells: 8) NSC, 9) NSC + Lac mice.

TABLE 1. Specificity of Suppression of Immune Response Mediated by Soluble Factors of ISC

Material for transfer into CP-recipients	Antigen for immunization	Antigen for immunization	
		to SRBC ($n=11$)	to RRBC ($n=10$)
NSC, $2 \cdot 10^7$	SRBC	2786 (1901÷4083)	—
NSC, $2 \cdot 10^7$	RRBC	—	1239 (760—2018)
NSC, $2 \cdot 10^7$ + SN	SRBC	248 (156—394)	—
NSC, $2 \cdot 10^7$ + SN	RRBC	—	1104 (723—1687)

*M_{geom} and confidence intervals at $P < 0.05$; n) number of mice.

After the ISC had been washed twice in medium 199 the concentration of the suspension was made up to $2 \cdot 10^8$ cells/ml and it was treated with ultrasound on an MSE apparatus (England) for 3 min during cooling, after which it was centrifuged at 20,000g for 30 min at 4°C. The effect of SN on the immune response was studied by the use of adoptive transfer of normal spleen cells (NSC) of intact mice together with SN into syngeneic recipients treated with cyclophosphamide (CP-recipients) in a dose of 200 mg/kg 4-5 h before transplantation of $2 \cdot 10^7$ NSC and SN into them. The CP-recipients were immunized intraperitoneally with SRBC in a dose of $2 \cdot 10^8$ cells 20-25 min after transfer of the NSC. The number of antibody-forming cells (AFC) in the animals' spleens was determined by the local hemolysis in agar method [6] on the 5th day after transfer of the cells and immunization.

TABLE 2. Effect of Heating to 56°C on Suppressor Activity of Factor from ISC and Serum of Immune Mice

Material for transfer	Number of AFC in spleen	Number of mice
NSC	4036 (2965–5495)	15
NSC + SN	658 (508–851)	16
NSC + SN, heated to 56°C	1156 (718–1862)	15
NSC + IS	114 (76–171)	15
NSC + IS, heated to 56°C	169 (79–363)	14

*M_{geom} and confidence intervals at $P < 0.05$.

EXPERIMENTAL RESULTS

In the experiments to study the specificity of suppression of the immune response by the factor contained in SN, after transfer of NSC and SN into the CP-recipients they were given an intraperitoneal injection of SRBC or of rat red blood cells (RRBC) in a dose of $2 \cdot 10^8$. It will be clear from Table 1 that SN suppressed the immune response to SRBC more than tenfold but did not affect the immune response to RRBC.

To determine the presence or absence of a receptor on the suppressor factor of ISC for SRBC, the SN was absorbed with SRBC, RRBC, and human (HRBC) and mouse (MRBC) red blood cells twice (for 45 min each time), using a red cell residue (40% relative to the volume of SN). It will be clear from Fig. 1 that only absorption with SRBC abolished the suppressor properties of SN.

The next step was to study whether syngeneic or allogeneic cells of an immunocompetent organ (spleen) or nonimmunocompetent cells (liver) could absorb the suppressor factor of ISC. In all experiments of this series SN was absorbed twice at room temperature for 45 min each time, using a compact cell residue amounting to 20% of the volume of SN. The initial concentration of ISC when SN was obtained was $2 \cdot 10^8$ cells/ml. In one experiment SN was absorbed with syngeneic mouse liver cells. It will be clear from Fig. 2a that activity of the suppressor factor of SN was unchanged after this treatment. Absorption of SN by both syngeneic and allogeneic spleen cells considerably reduced its suppressor activity (Fig. 2b).

The fraction of specific IgG contained in serum of immune mice and also, possibly, in SN, is known also to have the property of specifically suppressing the immune response. In no case did the titer of hemagglutinins and hemolysins in SN exceed 1:4-1:8. Nevertheless, the effect of IgG, even in low titer, on the immune response could not be completely ruled out. To investigate this problem experiments were carried out to compare some properties of the suppressor factors of SN and immune serum (IS). As Fig. 2c shows, unlike SN, IS did not lose its suppressor properties after absorption with syngeneic NSC. Heating SN to 56°C for 60 min led to considerable loss of suppressor activity, but similar treatment of IS did not change it (Table 2).

In the last series of experiments the ability of pronase (Serva) to inhibit the suppressor activity of SN was studied. For this purpose, pronase was added in a dose of 650 μg/ml to SN (the initial concentration of ISC when SN was obtained was $2 \cdot 10^8$ cells/ml) and incubated at 37°C for 45 min. SN obtained in the same way from NSC, and also treated with pronase, served as the control.

The results are evidence that in this model suppression of the immune response was due to soluble factors capable of suppressing the response only to the specific antigen. According to data in the literature, specificity of suppression is connected with the presence of a site (receptor) on the suppressor molecule which binds the antigen [5, 7, 13]. The same conclusion can be drawn from the results of the present experiments, for only absorption of SN with the specific antigen (SRBC) led to abolition of the suppressor effect.

The ability of spleen cells, but not liver cells, of both syngeneic and allogeneic mice to reduce the suppressor activity of SN deserves attention (Fig. 2a). The suppressor factor evidently carries a receptor for immunocompetent cells, irrespective of their H-2 genotype. It may be that the mechanism of suppression in this particular system differs from that in the model described by Tada et al. [11], for the suppressor factor in that model was accepted by T-cells of mice syngeneic with the donors of the factor with respect to region I-G of the H-2 complex. The possibility cannot be ruled out that acceptance of the suppressor factor on allogeneic target cells (T or B) may lead to specific suppression not restricted relative to the H-2 complex. Evidence in support of such a possibility is given by data in the literautre [8, 9, 13] and also by results obtained by the present writers in special investigations.

As regards the nature of the factor suppressing the immune response, it must be stated that it is evidently not in the IgG class. This is shown by differences in the properties of SN and IS (see Fig. 2 and Table 2). It can be tentatively suggested that this factor, like other antigen-specific immunoregulatory molecules [5, 7, 12], is a protein, for treatment with pronase considerably reduced its suppressor activity. Further investigations will be carried out to study the mechanism of action of the suppressor factor.

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