the cornea. It is possible that the cytokine-effected delay of fibrin resolution by macrophages at the earlier stages of healing results in a latter filling of the wound canal with newly formed scar tissue characterized by a higher keratoblast content.

The fact that cytokines affect the earliest stages of inflammatory and regenerative processes was confirmed by further experiments with cytokine complex administration one month after the injury. The preparation was used for a month without any effect on scar size being noted. Previously we demonstrated that the cytokine fraction of molecular weight 30-10 kD noticeably changed the metabolic profile of phagocytes and regulated their mobility, activized phagocytosis, potent biooxydant generation, production of immunopeptides (interleukin-1, tumor necrosis factor, etc.), and regulated fibroblast functions [6]. It may be assumed that the effect of the cytokine complex was manifested in a depression of fibroblast excessive proliferation and inflammatory reaction inhibition due to inhibition of macrophage and leukocyte migration toward the focus of injury from the limbic area. It is also possible that exogenously introduced cytokines induce immunopeptide production by the cells and normalize the healing processes.

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EXPERIMENTAL GENETICS

Characteristics of T-Suppressors Responsible for Cellular and Humoral Immune Response Competition in the Spleen

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The possibility of competence between delayed-type hypersensitivity (DTH) and antibody production (ABP) T-effectors at the stage of mature antibody-producing cells in the spleen has been previously demonstrated [1] with antigen-specific DTH T-suppressors acting as contrasuppressors with respect to the ABP productive phase in the spleen [1,3].

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The present research was aimed at the detection and functional assessment of splenocytes responsible for T-effector-dependent ABP production.

MATERIALS AND METHODS

A total of 720 CBA, DBA/2, and (CBA×C57Bl/6)F_1 hybrid mice weighing 18 to 22 g were used in the study.

Antibody production was induced by subcutaneous immunization into the foot with 1×10^8 sheep erythrocytes (SE). Counts of M or G antibody-producing cells (APC-M and APC-G) were determined, respectively, by direct [7] or indirect [8] local hemolysis 5 days after immunization in the regional (popliteal) lymph node. DTH was assessed as described previously [2] from the degree of foot swelling in response to repeated injections of SE (0.1 mm difference was taken as 1 U), the magnitude of nonspecific edema of the foot in response to primary injection of the antigen being taken as the zero reaction level.

The mice were intraperitoneally primed with SE in doses 1×10^5 to 1×10^8 for splenic suppressor cell (SSC) production and assessment; after 4 days, donor splenocytes (5×10^6 in 0.05 ml of medium 199) were subcutaneously transplanted to syngeneic recipients into the same foot in which they were immunized 4 days before by the method described above. SSC activity was established from the reduction of the antibody-producing cell count in the lymph node one day after their transplantation. Experimental methods differing from the described scheme will be presented separately.

In special experiments for the removal of adhering cells (macrophages) SSC-containing splenocytes were incubated in a concentration of 5×10^6/ml (3 ml of medium 199 with 20% embryonal calf serum in 3% CO_2 environment) in standard plastic dishes 90 mm in diameter. The remaining cells were then washed with medium 199 and transplanted to recipients in a dose of 5×10^6 taking into consideration the initial preincubation cell concentration. For the removal of T-lymphocytes SSC were incubated in the presence of the complement with anti-Thy-I serum [6] and the remaining splenocytes were transplanted in a dose of 5×10^6 according to the routine scheme. SSC antigen specificity was detected in experiments with intraperitoneal donor immunization with rabbit (1×10^7), rat (1×10^8), and allogenic (1×10^7) erythrocytes and recipient immunization with sheep erythrocytes. To check SSC restriction with recipient cells with respect to the H-2 histocompatibility antigen, SSC were induced in CBA mice ($H\text{-}2^k$) and their activity tested in DBA/2 ($H\text{-}2^d$) mice. SSC precursor sensitivity to cyclophosphamide was assessed by subcutaneous injections of the agent in doses of 20 or 200 mg/kg 2 days before intraperitoneal immunization of mice. T-suppressor generation in recipients was blocked by 20 mg/kg cyclophosphamide [1,9], while hyperfunction was blocked by intraperitoneal preimmunization (10 days prior to subcutaneous immunization) in a dose of 1×10^4 sheep erythrocytes. This SE dose was not immunogenic with respect to ABP or DTH [2] but it provoked T-suppressor generation [4]. Control mice were injected intact donor splenocytes in all cases.

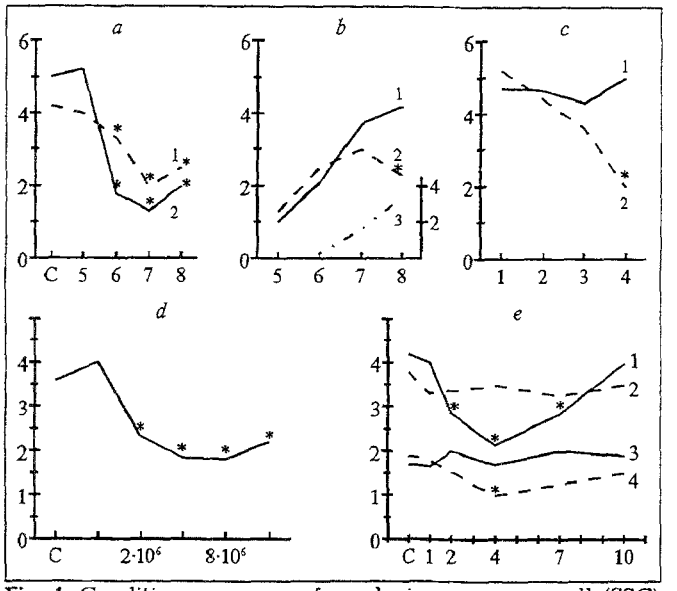

Fig. 1. Conditions necessary for splenic suppressor cell (SSC) generation. Plotted on the ordinate (here and in Fig. 2): number of antibody-producing cells ($\times10^3$) per lymph node; to the right on Fig. 1, *b*: DTH expression (in U) in recipient. Plotted on the abscissa: *a*) log of SE dose used for intraperitoneal donor immunization; *b*) log of SE dose used for recipient subcutaneous immunization; *c*) day after recipient immunization start on which SSC were transplanted to recipients; *d*) dose of transplanted SSC; *e*) days elapsed after donor intraperitoneal immunization till SSC transplantation; C: control (*a*, *d*, *e*). *a*: *1*) CBA mice; *2*) CBA×C57Bl/6)F_1 mice; *b*: *1*) control; *2*) SSC effect; 3) DTH expression; *c*: *1*) control; *2*) SSC effect; *e*: *1*) M-antibody-producing cell count 5 days after recipient immunization; *2*) G-antibody-producing cells 8 days; *3*) M-antibody-producing cells 8 days; *4*) G-antibody-producing cells 5 days after subcutaneous immunization. Asterisk indicates data significantly differing ($p<0.05$) from the control. Experimental series *b*, *c*, *d*, and *e* were carried out on CBA mice.

The results were statistically processed using Student's *t* test.

RESULTS

It is shown in Fig. 1 that the following conditions are necessary for SSC effect manifestation: 1) intraperitoneal immunization of donors with SE in doses of 1×10^6 or higher (Fig. 1, *a*); later SE in doses of 1×10^7 or 1×10^8 are used to induce SSC; 2) SSC are

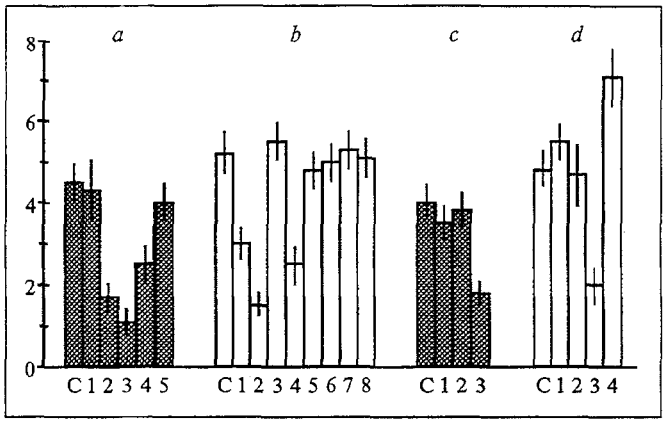

Fig. 2. SSC characteristics. C: control (*a, b, c, d*). *a*: *1*) injection of medium 199 instead of splenocytes to recipients; *2*) regular SSC transfer; *3*) SSC without cells adhering to plastic; *4*) SSC transfer in the presence of T–suppressor hyperfunction in recipients; *5*) T–suppressor hyperfunction simulation without SSC transfer; *b*: *1*) regular SSC transfer; *2*) SSC transfer during T–suppressor blocking in recipients by cyclophosphamide; *3*) injection of 20 mg cyclophosphamide without SSC transfer; *4*) injection of 20 mg.kg cyclophosphamide to donors; *5*) injection of 200 mg/kg cyclophosphamide to donors; *6*) injection of rabbit erythrocytes to donors; *7*) injection of allogenic erythrocytes; *8*) injection of rat erythrocytes; *c*: *1*) injection to DBA/2 mice of CBA intact splenocytes; 2) injection of CBA mice SSC; 3) syngeneic SSC transfer; d: 1) intravenous injection of suppressor splenocytes, 5×10^7; 2) parallel subcutaneous and intraperitoneal immunization with SE (1×10^8) of one and the same animal; 3) regular SSC transfer; 4) T cell removal from SSC. Experimental series *a* and *b* were carried out on CBA mice, *c* on DBA/2 mice, and *d* on (CBA×C57Bl/6)F_1 mice.

active only when the recipient is immunized with SE in the same doses that induce abundant production of not only antibody-producing cells but of T-effectors as well (Fig. 1, *b*); 3) these cells are active in the productive but not the inductive phase of ABP (Fig. 1, *c*); 4) the minimal effective SSC count is 2×10^6 per recipient (Fig. 1, *d*); 5) the time range of SSC activity manifests itself only at the peak of M- but not G-antibody response, that is, 5 but not 8 days after recipient immunization (Fig. 1, *e*). In addition, four-day SSC do not noticeably influence the counts of G-antibody-producing cells in the lymph node in CBA mice also during the period of the G-antibody response decrease 10 days after subcutaneous immunization of recipients (1800±250 in the control, 1600±190 in the experiment). This suggests that the suppression of G-antibody-producing cells on day 5 of the immune response (Fig. 1, *e*) is related not to a direct effect of SSC on these cells but to a reduction of the M-antibody-producing cell count or to a slowed rate of their switchover the production of G-antibodies. If, however, the counts of G-antibody-producing cells increase, the SSC effect on ABP is completely inactivated.

The functional characteristics of SSC are shown in Fig. 2: donor splenic suppressor activity is universally related to recipient T-suppressor activity (Fig. 2, *a, b*); intact (control) donor splenocytes do not noticeably influence ABP in recipient in comparison with that after injection of medium 199 alone (Fig. 2, *a*); removal of cells adhering to plastic from the total number of suppressor splenocytes does not reduce their activity (Fig. 2, *a*); the SSC effect is antigen-specific (Fig. 2, *b*), requires H-2 antigen complex restriction (Fig. 2, *c*), and does not manifest itself during SSC intravenous transfer or induction and assessment in a single animal (Fig. 2, *d*); SSC precursors are sensitive to 200 but not to 20 mg/kg cyclophosphamide (Fig. 2, *b*), this permitting their differentiation from T-suppressors, on the one hand, and from T-effectors, on the other [1,5,9]; the removal of T-cells from the pool of splenic suppressors gives rise to the opposite result: ABP stimulation (Fig. 2, *d*). ABP stimulation in such a case is not mediated by donor antibody-producing cells, because negligible counts of M-antibody-producing cells (80±17) are detected in the popliteal lymph nodes of intact recipient after splenocyte transfer from donors intraperitoneally immunized with 1×10^8 SE.

Hence, T-suppressors capable of inhibiting fully formed M-antibody-producing cells, depending on T-effectors activity, are accumulated in the mouse spleen during the immune response. Under ordinary conditions these cells are unable to home into the lymph nodes and seem to operate only in the spleen. After subcutaneous adaptive transfer, SSC can suppress ABP in the regional lymph node, a fact which makes it possible to use this phenomenon as a model for assessing splenic suppressor cells function in experiment.

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