EFFECT OF ANTIGEN-INDUCED SUPPRESSOR B CELLS ON DEVELOPMENT OF MEMORY B CELLS, CARRIER-SPECIFIC HELPER T CELLS, AND ANTIBODY-FORMING CELLS

A. G. Kalinkovich, E. L. Luganskaya, and B. V. Pinegin

UDC 612.112.94.017.1+612.112.94.017.4]-06-08

KEY WORDS: antigen-induced suppressor B cells, memory B cells, helper T cells, antibody-forming cells, Fc receptor.

The existence of suppressor B cells was first discovered in [3]. Further study showed that suppressor B cells can be induced by antigens, immune complexes, microorganisms, and polyclonal stimulators [1, 5, 10-12, 14, 15]. As regards antigen-induced suppressor B cells (AISB) we know that they are B cells which do not adhere to plastic, which act antigen-nonspecifically, and which are not H-2 restricted [1].

The aim of the present investigation was to study the presence of an Fc receptor (FcR) on the surface of AISB, their sensitivity to antiproliferative agents, their interaction with other immunoregulator cells and with precursors of antibody-forming cells (AFC).

EXPERIMENTAL METHOD

Experiments were carried out on (CBA × C57BL/6)F_1 mice of both sexes weighing 18-20 g, obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR. The mice received an intraperitoneal injection of $5 \cdot 10^7$ sheep's red blood cells (SRBC). Seven days later cells adherent to plastic were removed from the splenocyte suspension [1], after which a population enriched with B cells (AISB) was obtained from the nonadherent cells by the two-stage cytotoxic reaction with anti-Thy-1,2-serum and fresh rabbit complement.

In the experiments of series I, $25 \cdot 10^6$ AISB were injected intravenously into recipients simultaneously with immunization (day 0), and $5 \cdot 10^7$ SRBC were injected 5 or 14 days after immunization. On day 7 (for the first two groups) and day 16 (group 3) memory B cells (MB) were obtained, and injected in a dose of $5 \cdot 10^6$ together with 10^7 normal spleen cells, into recipients which had been irradiated 6 h previously in a dose of 8.5 Gy (^{137}Cs, dose rate 5.82 Gy/min) on the Stebel' 3a apparatus. The recipients were given an intraperitoneal injection of $2 \cdot 10^8$ SRBC 30-60 min later. On the 6th day the number of IgM-AFC in the spleens was counted by Jerne's method. They were also counted in spleens of the MB donor mice on days 7 and 16.

In the experiments of series II AISB were injected into recipients in the same dose and at the same times as in series I, but carrier-specific helper T cells (HT) were obtained from their spleens by the method described previously [9], using affinity-purified rabbit IgG against mouse immunoglobulins, mixed with 0.1% sodium azide (from Serva, West Germany). HT were injected in a dose of $5 \cdot 10^6$ into lethally irradiated recipients in the same syringe with 10^7 B cells from mice immunized twice, 8 and 4 weeks, respectively before the experiment, with 100 μg of a complex of trinitrobenzenesulfulfonic acid with rabbit gamma-globulin (TNS-RGG). The recipients were immunized 30-60 min later with a complex of TNS with $2 \cdot 10^8$ SRBC. Six days later the number of AFC against TNS, conjugated with horse red cells (HRBC) was determined in the spleens. In some experiments AISB were divided into FcR^+ and FcR^- cells. In this case the recipients each received $15 \cdot 10^6$ FcR^+ or FcR^- AISB. AISB (10^7/ml) also were treated *in vitro* for 30 min at 37°C with mitomycin C (Serva) in a concentration of 50 μg/ml or with cycloheximide (Serva) in a dose of 25 μg/ml, or the donors were irradiated in a dose of 8 Gy 4 h before the AISB were taken from them. Conjugates of TNS with RGG (epitopic density 22) [7] and with SRBC and HRBC 13 were prepared by the method described previously. The cells were separated into FcR^+ and FcR^- cells by the method in [9], using heat-aggregated human

Institute of Immunology, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR R. V. Petrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 7, pp. 58-60, July, 1986. Original article submitted February 12, 1985.

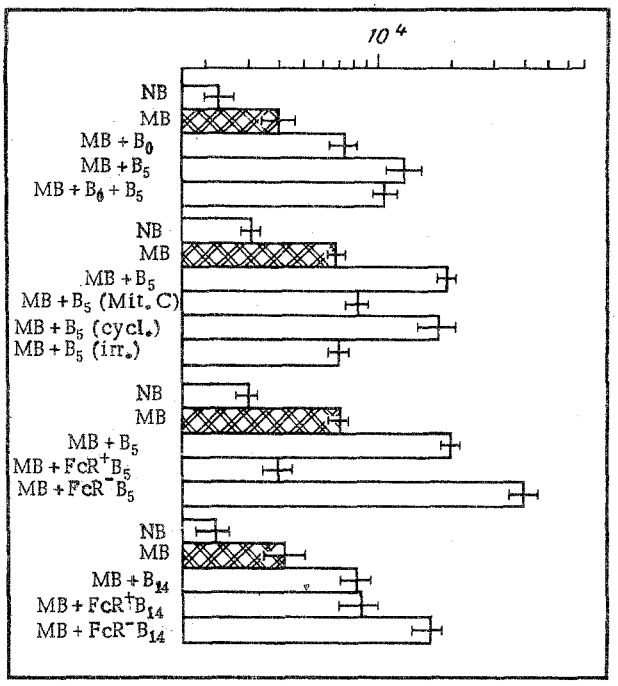

Fig. 1

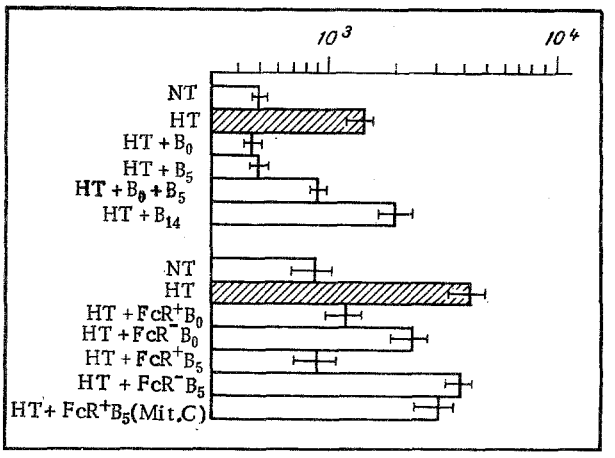

Fig. 2

Fig. 1. Effect of AISB on MB development. Horizontal axis — number of anti-SRBC AFC. NB and MB) Normal B cells (NB) or memory B cells (MB) injected into irradiated recipients. Here and in Figs. 2 and 3: B_0, B_5, and B_{14}) SRBC injected into unirradiated recipients and AISB were injected at the same time as (0) or 5 or 14 days later. Mit. C, Cycl., Irr.) AISB treated beforehand *in vitro* with mitomycin C or cycloheximide, and *in vivo* by irradiation, respectively. FcR^+ and FcR^-) AISB separated beforehand into FcR^+ and FcR^- cells.

Fig. 2. Effect of AISB on development of carrier-specific helper T cells. Horizontal axis — number of anti-TNS AFC. NT and HT) Normal T cells (NT) or carrier-specific helper T cells (HT) were injected into irradiated recipients.

serum IgG as the sorbent. The method of obtaining the anti-Thy-1,2-serum and its characteristics and the method of testing the purity of the populations obtained were described previously [1]. Dead cells were removed by the method in [6]. The viability of cells injected into the recipients was not less than 90%. All the experiments were repeated twice. At each point in one experiment seven to nine recipients were used. Values of AFC are given with confidence intervals at $P \leq 0.05$.

EXPERIMENTAL RESULTS

After one or two injections of unfractionated cells, and in particular, of FcR^- AISB, stimulation of MB formation was observed (Fig. 1). Preliminary treatment with mitomycin C and irradiation, but not with cycloheximide, abolished the stimulating effect of AISB. Conversely, FcR^+ AISB inhibited MB development but only if injected in the early stage of the immune response.

Unfractionated cells and FcR^+ AISB, injected in the early stages of the response, inhibited the formation of carrier-specific HT (Fig. 2) and AFC (Fig. 3). In both cases the FcR^- AISB had no suppressor activity. Treatment of FcR^+ AISB with mitomycin C almost completely abolished their suppression of HT development (Fig. 2). Meanwhile, the suppressor action of AISB on AFC formation was resistant to mitomycin C, irradiation, and cycloheximide (Fig. 3).

Since the suppressor effect of FcR^+ AISB was observed only in the early stages of the immune response taking place in the recipients, the most probable mechanism of their action may be their ability to inhibit proliferation of precursors of both immunoregulator (MB and HT) and effector (AFC) cells. In that respect they resemble bone-marrow suppressor B cells [2]. Meanwhile the difference in the sensitivity of AISB to the inhibitors used indicates heterogeneity of the suppressor action of AISB on precursors of HT and AFC and of the stimulating action on MB precursors. This last effect of AISB is associated with the fact that

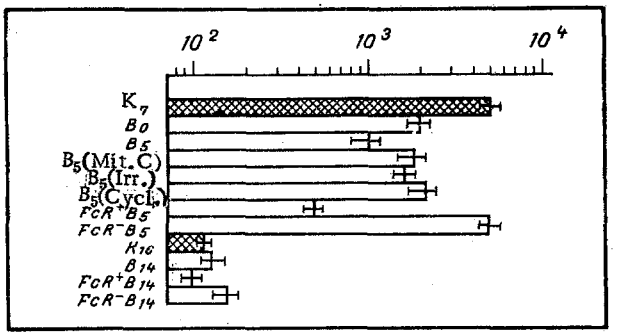

Fig. 3. Effect of AISB on development of AFC. K_7 and K_{16}) Number of AFC determined in recipients not receiving AISB 7 and 16 days, respectively, after their immunization (control). Remainder of legend as to Fig. 1.

they are evidently themselves MB precursors, because according to the experimental conditions AISB are B cells, adherent to plastic, and obtained 7 days after immunization of mice with a suboptimal dose of SRBC. The complex mechanism of interaction between AISB and precursors of various immunocompetent cells, and also the unequal sensitivity of the two effects of AISB to different inhibitors are reflected in the literature [4, 5, 8, 3, 15].

LITERATURE CITED

1. A. G. Kalinkovich, E. L. Luganskaya, and B. V. Pinegin, Immunologiya, No. 2, 21 (1984).
2. R. V. Petrov and R. M. Khaitov, Usp. Sovrem. Biol., 91, No. 1, 8 (1981).
3. R. V. Petrov, R. M. Khaitov, and A. A. Batyrbekov, Dokl. Akad. Nauk SSSR, 226, 1446 (1976).
4. V. M. Pisarev, N. N. Smirnova, and L. A. Pevnitskii, Byull. Éksp. Biol. Med., No. 9, 327 (1977).
5. C. E. Calcins, T. A. Stanton, and O. Stutman, Eur. J. Immunol., 10, 449 (1980).
6. W. F. Davidson and C. R. Parish, J. Immunol. Methods, 7, 291 (1975).
7. J. W. Goding, J. Immunol. Methods, 10, 61 (1976).
8. M. W. Kennedy and D. B. Thomas, J. Exp. Med., 157, 547 (1983).
9. M. G. Mage, L. L. McHugh, and T. L. Rothstein, J. Immunol. Methods, 15, 47 (1977).
10. M. Miyama-Inaba, T. Suzuki, Y. Paku, et al., J. Immunol., 129, 882 (1982).
11. U. Persson, J. Immunol., 118, 789 (1977).
12. R. V. Petrov and R. M. Khaitov, Cell. Immunol., 28, 298 (1977).
13. M. B. Rittenberg and K. L. Pratt, Proc. Soc. Exp. Biol. (N.Y.), 132, 575 (1969).
14. V. Rivas and T. J. Rogers, J. Immunol., 130, 376 (1983).
15. T. Shimamura, K. Hashimoto, and S. Sasaki, Cell. Immunol., 66, 104 (1982).