DIFFERENCES IN EXPRESSION OF DIFFERENTIAL ACTIVATED CELL ANTIGEN (ACA-1) ON T KILLERS AND MIF PRODUCERS

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Activation of lymphocytes is accompanied by changes in the antigenic properties of their surface. Special antigens of T killers [12] and plasma cells [14, 16], stimulated by mitogens of lymphocytes [2, 8, 10], have been found. A special differential ACA-1 antigen (activated cell antigen) was found previously with the aid of xenogeneic sera on the surface of T and B lymphocytes activated by various H-2 antigens, sheep, rabbit, and rat erythrocytes, and *Salmonella typhi* Vi antigen. This antigen was not found on intact T and B lymphocytes and differs from antigens hitherto known (H-2, Ig, Ala-1, Lyt 1, 2, 3, idiotype, Thy-1, MTL A, MBL A, etc.) [3-5]. However, in the investigations cited above the action of anti-ACA-1-antibodies on the functional activity of different subpopulations of activated T cells was not studied. It was shown previously that T killers and T cells producing macrophage migration inhibition factor (MIF) can be differentiated from one another by several properties [9, 11, 15].

The object of this investigation was to determine the presence of ACA-1 on these two subpopulations of T cells immune against H-2 antigens. For this purpose, suspensions of CBA mouse cells containing both T killers and T-MIF-producers simultaneously, were treated *in vitro* with anti-ACA-1 serum with complement and the ability of these cells to exhibit cytotoxic activity and to produce MIF was determined.

EXPERIMENTAL METHOD

CBA ($H\text{-}2^k$), AKR ($H\text{-}2^k$), C57BL/6 ($H\text{-}2^b$), BALB/c ($H\text{-}2^d$), and DBA/2 ($H\text{-}2^d$) mice weighing 18-20 g were used. The anti-ACA-1 sera were prepared and tested as described previously [3-5]. Antiserum against Thy-1,2 (anti-Thy-1,2) was obtained by repeated immunization of AKR mice with CBA thymocytes [13]. To induce T killers and T-MIF producers CBA mice were immunized by intraperitoneal injection of 20×10^6 EL-4 ($H\text{-}2^b$) or P-815 ($H\text{-}2^d$) tumor cells. The ability of the spleen cells of these mice to exhibit a cytotoxic action on the corresponding target cells or to produce MIF was studied 10-11 days after immunization. The cytotoxic action of the lymphocytes was determined from the release of ^{51}Cr from ^{51}Cr-labeled macrophages of C57BL/6 and BALB/c mice [1]. The cytotoxic effect was calculated by the formula:

$$\frac{O_{im}}{O_{max} - O_{norm}} \times 100\%$$

where O_{im}, O_{norm}, and O_{max} represent release of ^{51}Cr into the culture medium during incubation of target cells with immune and normal lymphocytes and with a 2% solution of sodium

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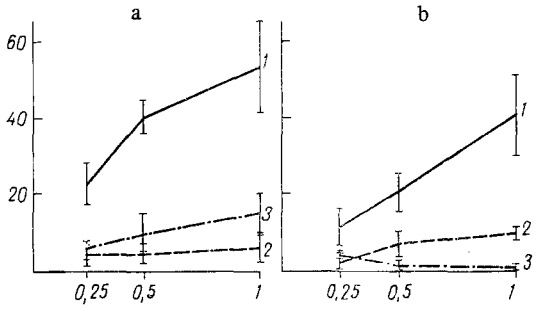

Fig. 1. Effect of anti-ACA-1-serum on T killers. Abscissa, dose of immune lymphocytes ($\times 10^6$) per well; ordinate, cytotoxic index. a) CBA-anti-EL-4 (H-2^k anti-H-2^b) lymphocytes; b) CBA-anti-P-815 (H-2^k anti-H-2^d) lymphocytes. Lymphocytes treated in the presence of complement with inactivated intact rabbit serum (1), anti-Thy-1,2 serum (2), or anti-ACA-1-serum (3).

dodecylsulfate respectively. Inhibition of macrophage migration was studied by the method described previously [6]. To estimate it quantitatively, the inhibition index (II) was determined by the formula:

$$(1 - A/B) \times 100\%,$$

where A and B are the mean zones of migration in the experiment and control respectively. Spleen cells of immune mice were treated *in vitro* with antisera and complement by a two-stage method: 0.6 ml of cells (4×10^7 to 6×10^7) in 1 ml were incubated with 0.2 ml antiserum at 4°C for 45 min. After centrifugation the cells were treated with 1 ml of rabbit complement (Cedarlane, Canada) in a dilution of 1:20 and incubated for 1 h at 37°C. The cells were then washed three times and tested for their ability to give a cytotoxic effect and to produce MIF. The results were subjected to statistical analysis by Student's t test. The results of three to five experiments are shown.

EXPERIMENTAL RESULTS

The results of the action of anti-ACA-1 serum on the cytotoxic activity of H-2^k anti-H-2^b and H-2^k anti-H-2^d killers are illustrated in Fig. 1a and b. Lymphocytes treated with inactivated intact rabbit serum induce marked lysis of the corresponding target cells (Fig. 1). Their cytotoxic action was indistinguishable from that of immune lymphocytes not treated with rabbit serum (data not shown). Anti-Thy-1,2-serum (Fig. 1:2) and anti-ACA-1-serum (Fig. 1:3) abolished the cytotoxic action of immune lymphocytes. Inhibition of the killer effect of anti-ACA-1-serum amounted to 70-80% and was independent of the specificity of the killers. Data on the effect of anti-ACA-1 serum on immune H-2^k anti-H-2^b cells producing MIF are given in Fig. 2. Treatment with intact rabbit serum (column 2) reduced the ability of the cells to produce MIF only very slightly (II = 47) compared with untreated cells (column 1) (II = 54). However, this inhibition was not statistically significant. Anti-Thy-1,2-serum (column 4) inhibited MIF production by 77-80% (II = 11.7). Anti-ACA-1-serum did not change the ability of the cells to produce MIF (column 3, II = 52.5).

It was shown previously that killers and MIF producers in the systems described are T cells [9]. These observations were confirmed in the present experiments: anti-Thy-1,2-serum abolished both the killer effect and MIF production. The effect of the anti-ACA-1-serum on these subpopulations of T lymphocytes was different: anti-ACA-1-serum effectively inhibited the cytotoxic effect but did not affect T cells producing MIF. The selective action of anti-ACA-1 antibodies on T killers was not associated with greater sensitivity of the T killers to treatment with antibodies and complement, for the T-MIF producers are more sensitive

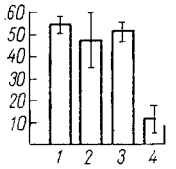

Fig. 2. Effect of anti-ACA-1-serum on T-MIF producers. Ordinate, II of macrophage migration. 1) Untreated CBA-anti-EL-4 (H-2^k anti-H-2^b) MIF producers, 2) treated in the presence of complement with inactivated intact rabbit serum, 3) with anti-ACA-1-serum, 4) with anti-Thy-1,2-serum.

than T killers to the action of anti-Thy-1,2-serum [9]. Activity of T killers of different specificity can thus be selectively inhibited by means of anti-ACA-1-serum without affecting the functioning of T-MIF producers. This is evidently due to the presence of ACA-1 on the surface of the T killers and the absence of this antigen on T-MIF producers. These facts directly confirm the previous indirect data on the difference between T-killers and T-MIF producers with respect to several properties [9, 11, 15], and they show that these cells are different subpopulations of T lymphocytes. The absence of ACA-1 on the surface of T-MIF producers may be connected with the fact that DNA synthesis and division are not required for MIF production in T cells [7]. Very probably ACA-1 appears only on cells which are in or have already passed through the S phase of the cell cycle.

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