PREPARATION OF MONOCLONAL ANTIBODIES TO ANTIGENS OF SPECIFIC MURINE SUPPRESSOR T CELLS

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Differential lymphocyte antigens provide a convenient model with which to study cell differentiation. Antigens specific for individual functional populations of T lymphocytes are particularly interesting. Some of these molecules are concerned with performance of their special functions by lymphocytes: LFA-1, Lyt-2,3, and L3T4 are responsible for the cytotoxicity of cytotoxic T lymphocytes (CTL) in mice [9, 11, 14], although they are found on a wider range of lymphoid cells. Other antigens serve as markers of several subpopulations; for example, OKT-5 and OKT-8 are represented on human suppressor T cells and CTL, whereas Lyt-2 and Lyt-3 are found in the same two T subpopulations of mice, and the 9.3 marker is found on human CTL [10].

The aim of this investigation was to obtain monoclonal antibodies (MCAB) interacting selectively with suppressor T cells.

EXPERIMENTAL METHOD

Specific suppressor T cells (SSTC) were induced in BALB/c ($H\text{-}2^d$) mice by intravenous injection of $9\cdot10^7$ C57BL/6 (B6) ($H\text{-}2^b$) spleen cells, irradiated in a dose of 1500 rads. On the 4th day SSTC from immune spleens were enriched by the adsorption – elution method on monolayers of $H\text{-}2^b$ macrophages [2], and $(MSU \times WAC)F_1$ rats were immunized twice with this population: $3\cdot10^7$ cells intraperitoneally, followed 14 days later by $2\cdot10^7$ cells intervenously. After a further 4 days, 10^8 rat spleen cells were hybridized, with the aid of polyethylene-glycol PEG-1500 (Schuhardt, West Germany) with 10^7 NS-1 mouse myeloma cells [5]. After hybridization the cells were transferred into 96-well plates (3040, Falcon, USA) in growth medium consisting of: DMEM with 15% embryonic calf serum, 4 mM L-glutamine (all from Flow Laboratories, England), 100 U/ml of gentamicin (from Farmakhim, Bulgaria), with the addition of 10^{-4} M hypoxanthine, $4\cdot10^{-7}$ M aminopterin, and $1.6\cdot10^{-5}$ M thymidine (all from Sigma, USA) – described as HAT medium. After 10-14 days antibodies in the culture fluids (CF), interacting with immune splenic BALB/c-anti-B6 lymphocytes, purified from erythrocytes and nonviable cells, and attached to the plate with poly-L-lysine (Sigma), were determined by radioimmunoassay [15]. Pure rabbit antibodies against rat immunoglobulins (Ig), absorbed with mouse Ig, were used as ^{125}I-labeled antibodies. The hybridomas were cloned by culture in 1.2% methylcellulose with 25% horse serum [5] or by the final dilutions method. MCAB accumulated in the CF or in ascites fluid (AF) of nu/nu mice, reared at the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR. Activity of SSTC, treated with mitomycin C (Sigma) and added to a one-way mixed lymphocyte culture (MLC), was estimated as the index of inhibition of ^3H-thymidine incorporation after incubation for 5 days [3].

CTL and producers of macrophage migration inhibition factor (MIF producers) were induced by intraperitoneal immunization of BALB/c mice with $2\cdot10^7$ EL-4 ($H\text{-}2^b$) leukemia cells and tested on the 10th and 15th day, respectively. The results were expressed as the cytotoxic index [4] and migration inhibition index [6]. To treat MCAB, 10^7 cells were incubated in 1 ml of

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TABLE 1. MCAB Used in the Work

Conventional name of MCAB	Specificity	Class, type of MCAB	Myeloma	Source of immune spleen cells
C-1	SSTC	μ, K	NS1	(MSU × WAC)F_1 Rats
C-4	SSTC	μ, K	NS1	(MSU × WAC)F_1 Rats
NATF9.9 [7]	Lyt-3,2	μ, K	X 63.653	AKR Mice

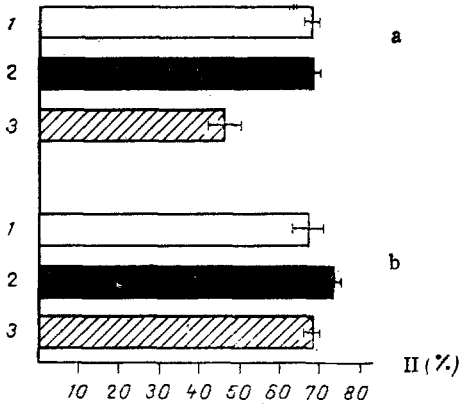

Fig. 1. Action of MCAB *in vivo*. Vertical axis — nu/nu mouse serum, AF with C1 MCAB (2) or C4 MCAB (3); horizontal axis — index of inhibition of DNA synthesis (in %). A) Injection 2 days, and B) 4 days after intravenous immunization of mice. Mean values with error (n = 4) are shown.

whole CF or AF, diluted 1:100 in medium RPMI-1640 with 10 mM HEPES (Flow Laboratories), and in the controls, in growth medium or nu/nu mouse serum at 20°C for 45 min, centrifuged, incubated in 1 ml of rabbit complement (Cedarlane, Canada) in a dilution of 1:15 at 37°C for 60 min, and then washed off twice. The number of living cells was then counted and they were used in the corresponding test. In some experiments, to inactivate suppressors, immune (normal in the control) lymphocytes, treated with mitomycin C and transferred at the rate of $5 \cdot 10^5$ cells per well into 96-well plates with V bottom (IS-MVC-9GTC, from Linbro, England), were centrifuged, 20 μl of AF or normal nu/nu mouse serum and 1 μl of whole complement were added to the residue, and the samples were incubated for 45 min at 37°C and washed twice. Next, 200 μl of a mixture of reacting and stimulating lymphocytes ($3 \cdot 10^5$ and 10^6, respectively) was added to each sample.

To assess the effect of MCAB *in vivo* mice were injected with 50 μl AF or normal nu/nu serum together with 100 μl of rabbit complement 4 days after allogeneic immunization, or without complement 2 days before immunization. The class and type of the MCAB and their concentration were determined by gel filtration and inhibition of radioimmunoadsorption [13].

EXPERIMENTAL RESULTS

Growth of hybrid cells was observed in 75% of the wells and in 30% of wells antibodies to mouse lymphocytes were found. The positive cultures were cloned and the clones were reared as lines. Twelve lines of hybridomas, producing antibodies binding with lymphocytes, were obtained. These antibodies were tested for their ability to abolish the specific functions of T lymphocyte subclasses immune to antigens of the H-2 complex, SSTC, CTL, and MIF producers. Only two variants of antibodies, namely C-92- (C1) and C-4-20 (C4) (Table 1) inactivated suppressor T cells by 40-50%, but did not affect activity of the CTL and MIF producers (Table 2). C1 and C4 MCAB had no effect on CTL irrespective of the effector to target ratio (30:1, 10:1, 3:1), whereas anti-Thy-1,2-serum inactivated CTL even in the maximal dose. The incomplete abolition of the effect of C1 and C4 MCAB on SSTC may be due to expression of the corresponding determinants only on some SSTC, the heterogeneity of the SSTC as regards sensitivity to treatment with MCAB and complement, or low affinity of the antibodies. MCAB against Lyt-3,2-antigen completely abolished the action of SSTC.

TABLE 2. Effect of MCAB on Activity of T Cell Subpopulations of BALB/c Mice Immunized *in vivo* with C57BL/6 Cells

MCAB	Inhibition index (M ± m), %	Abolition, %	Cytotoxic index (M ± m), %	Abolition, %	Migration inhibition (M ± m), %	Abolition, %
Control	49,9±5,9 (3+3)	—	57,3±6,8 (2+2)	—	68,8±5,9 (2+0)	—
C-1	26,5±4,8 (3+3)	45,6	55,5±4,2 (2+2)	4,6	70,4±6,4 (2+0)	0
C-4	19,9±3,4 (3+3)	55,8	58,7±4,9 (2+2)	0	69,05±7,9 (2+0)	0
NATF9.9	—22,0	100	—		—	

Legend. Control — growth medium in experiments with CF and nu/nu mouse serum in dilution of 1:100 in experiments with AF. Number of experiments with CF (1st number) and AF (2nd number) shown in parentheses.

Since antisuppressor rat serum [1] abolishes the action of SSTC when injected *in vivo* 2 h before removal of the spleen from immune mice, and stimulates nonspecific T cells if injected 2 days before immunization, the effects of C1 and C4 MCAB were studied under the same conditions. AF or nu/nu mouse serum was injected into the mice in a dilution of 1:4 (Fig. 1). C1 MCAB had no effect on SSTC in any single case, whereas C4 MCAB depressed the inhibition index when injected on the day of the test, although less strongly than in the case of treatment of SSTC *in vitro*. C4 MCAB thus reproduce one of the effects of antisuppressor serum. This indirectly confirms the view that these effects are due to antibodies of different specificity [7]. The absence of effects of C1 MCAB may be due to the large number of factors affecting behavior of antibodies *in vivo* [12].

Although the connection between the antibodies thus obtained and other markers of suppressor T cells which have been described (Lym 22), for example), is not clear, they can be used for isolation, enrichment, and selective inactivation of suppressor T cells and to study the nature of the corresponding determinants.

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