Monoclonal Antibodies to LFA-I Molecule Beta-Chain Promote a Rise in the Cytotoxic Index of Effector cells and Stimulate their Proliferation

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Successful interaction of a T cell with antigen (AG) requires, besides a T-cell receptor (TCR), the expression of a number of coreceptor molecules which provide the binding (formation) of the AG-MHC protein complex (MHC - main histocompatibility complex) and signal transduction into the cell [8]. Specifically, AG-specific activation involves an adhesive molecule LFA-I (lymphocyte function-associated antigen) which is not directly bound to TCR. This molecule belongs to the family of integrins, heterodimers with non-covalently bound α- and β-chains. These transmembrane proteins are associated with the cytoplasmic domains of the cytoskeleton. By interacting with the ligand ICAM-I (intercellular adhesion molecule), LFA-I provides an additional adhesive mechanism necessary to the cell for implementing some other interactions such as MHC/AG-receptor, TCR, cytokines, etc. [6]. It is known that *in vitro* antibodies to LFA-I α-chain suppress a proliferative response in a mixed culture of lymphocytes (MCL) [9], T-cell proliferation in response to phytohemagglutinin (PHA) [4], concanavalin A, production of interleukin-2, and the T-cell-mediated antibody response [13], and also inhibit the lytic function of effector cytotoxic T lymphocytes (CTL) [15]. At the same time, interaction between LFA-I and ICAM-I directly promotes the development of the response of activated CTL [6,9,14]. On the other hand, antibodies to LFA-I β-chain produce a costimulating effect on T cells (together with anti-CD3 and/or CD2 antibodies) inducing a release of ca, lymphokine production, and cell proliferation [12-14]. However, there are indications that antibodies to the β-chain do not affect the cytotoxic activity of CTL [11]. The present study was undertaken to assess the effect of monoclonal antibodies (mAb) to LFA-I β-chain on effector CTL in separate tests for their cytotoxicity and proliferative reaction.

MATERIALS AND METHODS

The experiments were performed on mice of the following inbred lines at the age of 5-6 months: B10.D2(R101) with haplotype H-2K^{d}I^{d}D^d, C57Bl/6 (B6) (H-2K^{b}I^{b}D^b), and B10.M (M) (H-2K^{f}I^{f}D^f). The rats were obtained from the vivarium of the Cancer Research Center, Russian Academy of Medical Sciences. EL-4 tumor cells from B6 mice were cultivated as ascites by weekly passages on syngeneic mice. To induce primary effector CTL specific to K^b, R101 mice were immunized with EL-4 thymoma cells (2.5×10^7 per mouse, intrap-

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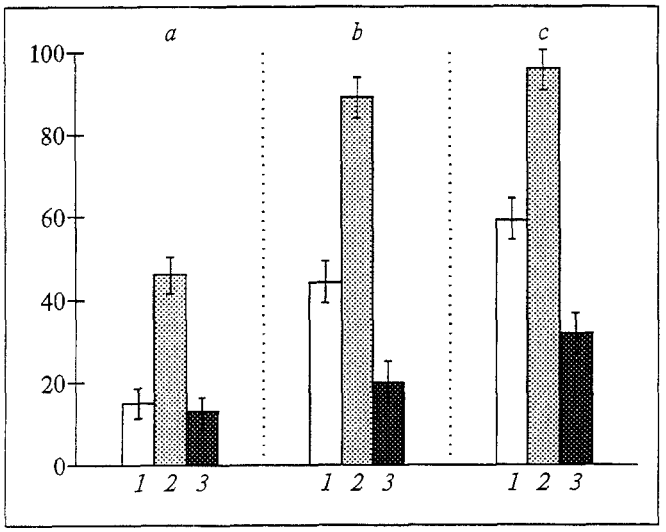

Fig. 1. Effect of mAb to LFA−I on cytotoxicity index (CI) of effector lymphocytes. Ordinate: CI, % of immune splenocytes eluted from R101 (*a*), M (*b*), and B6 (*c*) macrophages and treated with medium (*1*), anti−LFA−I mAb (*3*), and mAb to LFA−I β−chain (*2*). Here and in Fig. 2 the data of 4 experiments are presented.

eritoneally). After 11 days the immune splenocytes were enriched and fractionated on the basis of their adsorption on macrophage monolayers from B6 donor, third-party line M, and R101 recipient during 2 h at 37°C. After the removal of unattached lymphocytes, the adsorbed cells were twice eluted with a pronase solution (25 and 100 μg/ml) (Calbiochem) in the presence of pancreatine (Sigma) at the same concentration. The pronase was removed and inactivated with 30% cattle serum, and the lymphocytes were washed three times and incubated for 1 h at 37°C in a 5% CO_2 atmosphere. Subsequently, the cells were treated with a culture medium containing mAb to LFA-I [10] or to its β-subunit (kindly provided by Dr. Pierres, France) and incubated under the same conditions for 45 min. In the cytotoxicity test, ^{51}Cr-labeled mice fibroblasts (Ltk$^-$ of haplotype H-2^{k0} and their transfected derivatives 1-4 expressing the K^b-molecule [1] were used as targets. The target cells were applied to flat-bottom 96-well plates (Linbro) (10^4/well), twice washed before testing, and incubated with different fractions of immune lymphocytes for 16 h. The reaction medium was RPMI-1640 containing 100 U/ml gentamicin, 5% heat-inactivated fetal calf serum, 2 mM L- glutamine, and 10 mM HEPES. The cytotoxicity index (CI) was assessed from the equation: $CI=(a-c)/(b-c)\times100\%$, where *a*, *b*, and *c* reflect the experimental, maximal (in the presence of 1% Triton X-100), and spontaneous release of ^{51}Cr into the medium, respectively (cpm) [4]. In the experiments with creating a mixed lymphocyte culture (MLC), immune reacting splenocytes (5×10^5) and stimulators (10^6) irradiated with 2000 rad were mixed in 200 μl of complete growth medium for MLC: RPMI-1640 with 5% heat-inactivated human serum, 2 mM L-glutamine, 100 U/ml gentamicin, 5 mM 2-mercaptoethanol, and 25 mM HEPES and placed into 96-well round-bottom plates. Subsequently, 200 μl of RPMI-1640 with 0.04 MBq ^3H-thymidine (specific activity 1 Ci/mM) were added to each well. After a 16-h incubation at 37°C in a 5% CO_2 atmosphere, the cells were transferred onto fiberglass filters and incorporation of the radionuclide was assessed in a liquid scintillation counter. The results were expressed as the stimulation index SI=*a*:*b*, where *a* is the label incorporation into allogenic MLC after treatment of reacting CTL with antibodies; *b* is the label incorporation in MLC without antibody treatment. Cells were transferred onto the filters with a Cell Harvester (Skatron).

RESULTS

In the cytotoxicity test we used CTL-I fractionated on donor, third-party, and recipient macrophage monolayers and treated with mAb to the LFA-I molecule or to its β-subunit. It was demonstrated that antibodies to LFA-I that served as a positive control for function inhibition blocked the lysis of target cells (Fig. 1, *3*). These observations are in agreement with earlier reported characteristics of these antibodies [3]. On the other hand, mAb to LFA-I β-chain induced the opposite effect. They promoted lysis of target cells by effector CTL

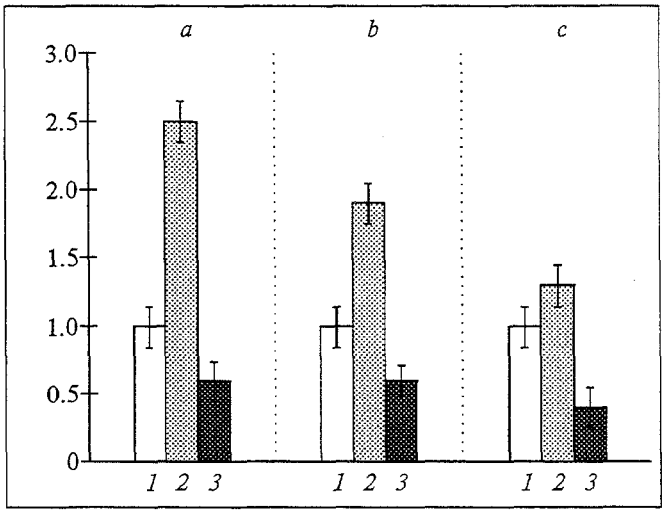

Fig. 2. Effect of mAb to LFA−I on stimulation index (SI) of immune splenocytes. Ordinate: SI (in absolute units) of effector T lymphocytes after elution from R101 (*a*), M (*b*), and B6 (*c*) macrophages and treatment with medium (*1*), anti−LFA−I mAb (*3*), and mAb to LFA−I β−chain (*2*).

eluted from recipient, third-party, and donor macrophage monolayers 4.1, 2.5, and 1.5 times, respectively (Fig. 1, *2*).

In the proliferative test we used the same CTL-I fractions preliminarily treated with mAb to LFA-I (as a positive control for inhibition) or to its β-chain. Irradiated splenocytes of the B6 donor line were taken as stimulators. As was expected, mAb to LFA-I inhibited to a certain extent the proliferative reaction of immune splenocytes in each of the three CTL-I fractions (Fig. 2, *3*). On the other hand, antibodies to the β-subunit stimulated proliferation of CTL-I eluted from recipient, third-party, and donor macrophage monolayers 2.5, 1.9, and 1.3 times, respectively (Fig. 2, *2*). A similar effect of proliferation enhancement has been described elsewhere for other antibodies acting together with mAb to LFA-I β-chain [12-14].

The results obtained attest that in the absence of other antibodies, mAb to LFA-I β-chain may produce a stimulating effect (or a costimulating effect if the presence of alloantigen is taken into account) on effector CTL-I in different test systems (cytotoxicity and proliferative tests). It is quite probable that this effect is of a nonspecific character, since the maximal stimulation was registered for immune T-lymphocyte fractions eluted from syngeneic and third-party macrophage monolayers (4.1 and 2.5 times for the CT test and 2.5 and 1.9 times for the proliferative test, respectively. The minimal effect (1.5 and 1.3 times, respectively, for the CT test and the proliferative test) was recorded for the CTL-I fraction eluted from the donor macrophage monolayer. It still remains unclear whether or not there is a connection between the proliferative reaction of CTL-I fractions and their cytotoxic function for treatment with mAb to LFA-I β-chain.

REFERENCES

1. H. Allen, J. Fraser, D. Flajer, *et al., Proc. Nat. Acad. Sci. USA*, **83**, 7447 (1986).
2. B. Brondz, I. Egorov, and G. Drizlikh, *J. Exp. Med.*, **141**, 11 (1975).
3. C. Desroches, D. Rigal, and C. Andrioni, *Scand. J. Immunol.*, **33**, 277 (1991).
4. G. Drizlikh, A. Andreev, I. Otomina, *et al., J. Immunol. Methods*, **8**, 383 (1975).
5. M. Dustin and T. Springer, *Ann. Rev. Immunol.*, **9**, 27 (1991).
6. M. Hibbs, H. Xu, *et al., Science*, **251**, 1611 (1991).
7. J. Klein, *Natural History of the MHC*, New York (1986).
8. A. Krensky, F. Sanchez-Madrid, E. Robbins, *et al., J. Immunol.*, **131**, 611 (1983).
9. M. Makgoba, M. Sanders, and S. Shaw, *Immunol. Today*, **10**, 417 (1989).
10. *Mechanisms of Cell-Mediated Cytotoxicity*, Eds. W. Clark and P. Golstein, New York (1982).
11. F. Sanchez-Madrid, P. Simon, S. Thompson, *et al., J. Exp. Med.*, **158**, 586 (1983).
12. G. Van Seventer, V. Shimizu, K. Horgan, *et al., Europ. J. Immunol.*, **21**, 1711 (1991).
13. T. Springer, *Nature*, **346**, 425 (1990).
14. T. Springer, *Ann. Rev. Cell Biol.*, **6**, 359 (1990).
15. C. Ware, F. Sanchez-Madrid, A. Krensky, *et al., J. Immunol.*, **131**, 1182 (1983).