MICROBIOLOGY AND IMMUNOLOGY

Monoclonal Antibody to the β-Chain of the LFA-1 Molecule Can Enhance the Cytotoxicity of Immune Splenocytes by Stimulating Their Proliferative Response

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Key Words: *LFA-1 molecule; T-cell proliferation; anti-LFA monoclonal antibodies; cytotoxicity*

By binding to the ICAM-I ligand, the LFA-1 molecule provides an additional mechanism of cell adhesion, thereby paving the way for a number of other interactions [3, 4]. The monoclonal antibody (mAb) to the β-chain LFA-1 can abrogate cell adhesion by specifically blocking the functional LFA-1 molecule, which is manifested in inhibited function of cytotoxic T lymphocytes (CTL) [9] or in inhibited proliferative response of activated lymphocytes [2,5]. A characteristic feature of this mAb (referred to henceforth as anti-β-chain mAb) is its costimulating action on T cells with anti-CD3 and/or anti-CD2 mAb, which leads to the release of Ca^{2+}, lymphokine production, and cell proliferation [6-8]. We have shown previously that anti-β-chain mAb alone (without costimulation by other antibodies) is capable of augmenting the immune response of fractionated splenocytes, thereby increasing their cytotoxicity index as well as their proliferative response [1]. However, there remained the question of how the proliferative response of these splenocytes relates to their cytotoxic function following their stimulation by anti-β-chain mAb. This is the first in a series of studies designed to answer this question.

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MATERIALS AND METHODS

Three strains of inbred mice from the vivarium of the Cancer Research Center (Moscow) were used: B10.D2(R101) with the H-2 haplotype $K^dI^dD^d$, C57BL/6(B6) (H-$2K^bI^bD^b$), and B10.M(M) (H-$2K^fI^fD^f$). The induction of primary effector CTL, separation of immune splenocytes into fractions by absorption-elution, and tests for cytotoxic and proliferative response were carried out as described previously [1]. The cytotoxicity of proliferating splenocytes was evaluated by incubating them with their target cells for 6 h.

RESULTS

Two main explanations may be offered to account for the enhanced cytotoxicity imparted to immune

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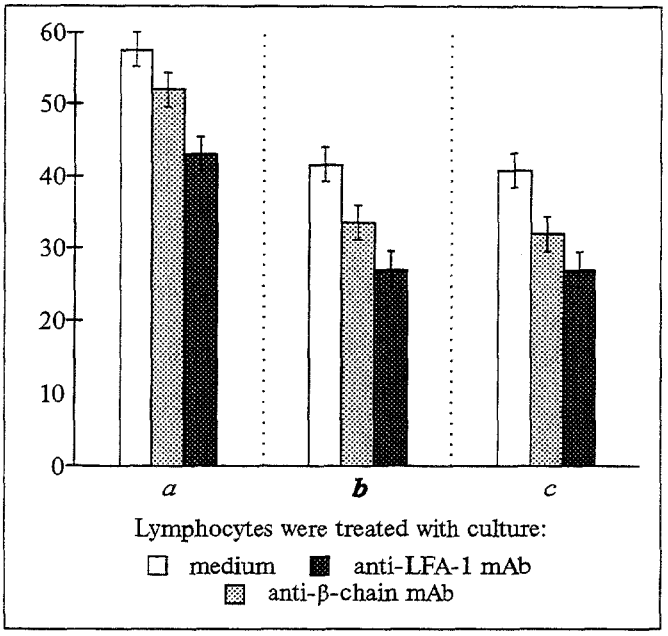

Fig. 1. Effect of anti-LFA-1 and anti-β-chain mAb on cytotoxicity index (CI) of immune splenocytes nonadherent to various macrophage monolayers. Ordinate: CI (in %) of cells nonadherent to recipient (*a*), third-party (*b*), or donor (*c*) macrophage monolayers. The data are means of four replicate tests.

splenocytes by anti-β-chain mAb: 1) stimulation by this mAb increases the proportion of effector cells in the test population as a result of their proliferation, or 2) enhanced cytotoxicity results not so much from an increase in the number of effector splenocytes as from the activation of already existing splenocytes with a consequent increase in their killer potential. It was therefore seemed important to compare the cytotoxicity indexes of immune splenocytes (both treated and untreated with anti-β-chain mAb) with those of the same cells after their proliferation in a mixed lymphocyte culture (MLC) for 16 h. Proliferation *per se* is not a sufficient condition for increasing the proportion of effector cells in the population: such an increase also depends on the proportion of effector splenocytes relative to other proliferating splenocytes present in the culture initially. For this reason, we initially used splenocyte fractions eluted from donor, third-party, and recipient macrophage monolayers, which resulted in different proportions of effector cells in these three fractions [1]. In tests with immune splenocytes that had not adhered to the macrophage monolayers, anti-β-chain antibody not only failed to stimulate these cells but even inhibited their function slightly (Fig. 1, *2*). As seen in this figure, a stronger inhibitory effect was exerted by anti-LFA-1 mAb which was used in control tests.

Pretreatment with anti-β-chain mAb of the immune splenocytes eluted from the macrophage monolayers and then placed in the MLC led to similar increases in their cytotoxicity regardless of the fraction to which they belonged (Fig. 2, *3*). In contrast, pretreatment of these cells with anti-LFA-1 mAb had little effect on their cytotoxicity (Fig. 2, *4*). It should be noted that during the incubation of immune splenocytes in the MLC without added antibody, their cytotoxicity indexes increased to become considerably higher than those of immune splenocytes that had not been stimulated in the MLC (cf. *2* and *1* in Fig. 2). If this had not happened, the role of natural proliferation in the MLC following the enhancement of effector splenocytes' cytotoxicity under the action of anti-β-chain mAb could be regarded as being insignificant. The present results did not allow us to decide whether the increase in the proportion of effector cells in the population was mainly due to their enhanced proliferation or to their further differentiation and/or activation as a result of stimulation with anti-β-chain mAb.

Further experiments are therefore required using, at the very least, cells of a particular phenotype ($CD8^+CD4^-$) and inhibitors of proliferation; also, the time during which the effectors are incubated with their target cells should be reduced to a minimum. Such experiments are now in progress.

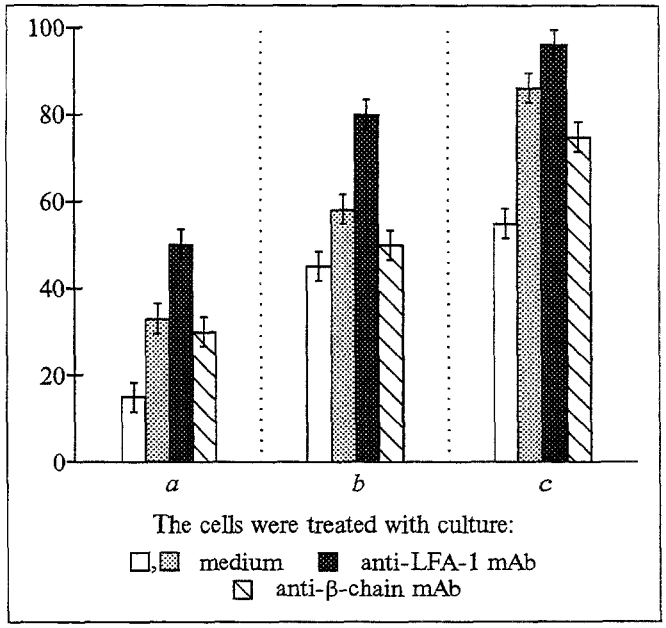

Fig. 2. Effect of anti-LFA-1 and anti-β-chain mAb on cytotoxicity index (CI) of fractionated effector splenocytes after their proliferation in MLC. Ordinate: the CI (in %) of splenocytes eluted from recipient (*a*), third-party (*b*), or donor (*c*) macrophage monolayers. Cells in (*1*) had not been placed in MLC. The effector to target cell ratio was 90:1. The data means of four replicate tests.

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Enhanced Production of Lymphocyte Alloimmune Antibodies After Plasmapheresis

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Plasmapheresis is used to remove from the body toxic substance which entered the organism with food [3] or drug overdose [12], endogenously forming pathological macroglobulins [6], pathological clone cells or fragments thereof [7], to remove cholesterol in hypercholesteroemia [8], immune complexes [9,10], and antiD antibodies [11], and in other similar clinical situations. However, the range of clinical applications of apheresis is not confined to the above cases. There are grounds for proposing that plasmapheresis be used to stimulate immune antibody production. Previous experiments with mice and rats have demonstrated that regeneration of some organs, namely, the liver and kidneys, after resection is associated with increased production of immune antibodies [1-3]. The present work was aimed at the investigation of lymphotoxic alloimmune antibody production under the action of plasmapheresis.

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MATERIALS AND METHODS

Plasmapheresis sessions were administered to hematological patients with, mainly, aplastic anemia, myelodisplastic syndrome, and some other diseases; the patients were sensitized to HLA by repeated transfusions of blood and its components (platelets). Removal of antiHLA antibodies directly before the next transfusion of blood components to prevent nonhemolytic posttransfusion reactions was an indication for apheresis, Plasmapheresis sessions were carried out using K-26D, PC-6 refrigerator with plasticized hemacon 500/300 sacs with gluguicir 1000 and imported sacs with citrate solution ACDA-600. The volume of plasma removed was 1/4 and 1/2 of the total circulating plasma volume. It was replaced with quick-frozen plasma, normal saline, and albumin. The patients were administered 5 to 10 sessions with intervals of 4 to 7 days.

The lymphocytotoxic test was the basic method for antibody assessment [5,13]. Each patient's sample was examined with lymphocytes of several donors. Lymphocytes were isolated from fibrin-free

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