Antibodies directed at mouse IL-2-R α and β chains act in synergy to abolish T-cell proliferation in vitro and delayed type hypersensitivity reaction in vivo

Abstract The anti-mouse IL-2-R β chain mAb TM-β1 which, by itself, does not affect IL-2-dependent proliferation through the high affinity mouse IL-2 receptor, was shown to cooperate in a synergistic way with a set of anti-IL-2-R α chain mAbs both in vitro and in vivo. In vitro, when associated at equimolar concentrations, the TM-β1/anti-α mAb association was four to ten times more efficient at inhibiting the proliferation of the CTL-L2 cell line than was a similar concentration of anti-α mAb alone. In addition, a bispecific antibody in which a Fab’ fragment of TM-β1 was covalently linked to a Fab’ fragment of one of the anti-α mAb (5A2) was shown to be as efficient as the TM-β1/5A2 association. The association of TM-β1 with 5A2 was also tested in vivo in a sheep red blood cell-induced delayed type hypersensitivity (DTH) model. TM-β1 which, by itself, had no effect on DTH, induced a two- to threefold decrease in the doses of 5A2 required to suppress this cell-mediated immune reaction.

Key words Monoclonal antibodies, mouse interleukin-2 receptor · Mouse, interleukin-2 receptor, monoclonal antibodies · Interleukin-2 receptor, monoclonal antibodies, mouse

Introduction

Interleukin-2 receptors (IL-2-R) comprise at least three membrane anchored glycoproteins [10]. These chains can combine in various ways to form receptors with different affinities for IL-2: the α chain (or CD25, Tac antigen) [12] is a low-affinity receptor ($K_d = 10^{-9}$ M), the β/γ complex is an intermediate-affinity receptor ($K_d = 10^{-9}$ M), and the α/β/γ complex is a high-affinity receptor ($K_d = 10^{-11}$ M). The γ chain [18] alone or in the presence of α chain does not bind IL-2. The β chain [5] alone binds IL-2 with very low affinity ($K_d > 10^{-7}$ M) and the α/β complex can form a pseudo high-affinity receptor ($K_d = 10^{-10}$ M). Of these various receptor forms, only two are biologically functional – the β/γ and α/β/γ receptor complexes – as one might expect given the fact that heterodimerization of β and γ chains is required for signal transduction [11].

Various therapeutic strategies have been developed that involve agents that eliminate IL-2-R expressing cells and/or block IL-2 interaction with its receptors [14, 22]. Among them are anti-α chain monoclonal antibodies (mAbs), which have been successfully used in organ transplantation [8, 9, 15, 16]. Anti-β chain mAbs have more recently been obtained that inhibit IL-2 binding to the β/γ complex. However, none of these antibodies were able to affect IL-2 induced proliferation through high-affinity α/β/γ receptors [4, 6, 17, 20]. We previously showed that combining anti-human α and anti-human β mAbs resulted in a high synergistic blocking effect on IL-2 high affinity binding and IL-2 induced T cell proliferation of human T lymphocytes [1]. On the basis of these results, we then constructed a bispecific antibody carrying the anti-α and anti-β valencies. As a result of α and β chains crosslinking, the bispecific mAb displayed a high affinity binding that was specific.
Construction of the bispecific antibody

This was performed essentially as described previously [4]. Briefly, Fab'2 fragments of 5A2 and TM-β1 mAbs were prepared by pepsin treatment and purified by gel filtration on a prepacked Sephadex G200 column (Pharmacia, Uppsala, Sweden). They were then reduced by treatment with 2-mercaptoethanol (MCEA) and sodium arsenite and reacted with 5-5'-dithiobis-2-nitrobenzoic acid (DTNB). The Fab'-TNB fragments obtained were separated by gel filtration and stored. The Fab'-TNB fragment obtained from 5A2 was reduced with MCEA to generate Fab'-SH. After removal of excess MCEA by gel filtration, it was mixed with an equimolar amount of Fab'-TNB prepared from TM-β1. The bispecific 5A2/TM-β1 antibody generated was purified from residual undigested IgG and unreacted Fab' fragments by gel filtration on a prepacked Sephadex G200 column (Fig. 1A). The purity of the bispecific antibody was further analyzed by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) under nonreducing conditions. A major band at 110 kDa was observed (Fig. 1B).

Fig. 1 A Gel filtration chromatogram showing the separation of TMβ1/5A2 bispecific antibody (peak 1) from undigested IgG (peak 2) and unreacted Fab' fragments (peak 3). B SDS-PAGE analysis (nonreducing conditions). From left to right molecular weight markers (in kDa), TM-β1 Fab'2 fragments, 5A2 Fab'2 fragments, and TM-β1/5A2 bispecific antibody

Materials and methods

Monoclonal antibodies (mAbs)

Hybridoma producing 125, 135, and 5A2 mAbs were a kind gift from J. Theze (Institut Pasteur, Paris, France) [2], and the mAbs were purified from ascitic fluids produced in nude mice. The TM-β1 mAb was prepared as described previously [19].

for α + β + activated T cells as compared to β + or α + cells. In addition, this bispecific antibody was shown to inhibit IL-2-induced proliferation at concentrations much lower than did the parental mAbs [4].

In this paper, using mAbs directed against the α and β chains of the murine IL-2-R, we have analyzed whether anti-α and anti-β mAb combinations and anti-α/anti-β bispecific mAbs could have interesting immunosuppressive properties both in vitro and in a delayed type hypersensitivity (DTH) model in vivo.

Results

Antiproliferative effects of the anti-murine IL-2-R mAbs

The antmurine IL-2-R α chain mAbs 5A2, 125, and 135 induced a dose-dependent inhibition of the IL-2-driven proliferation of the CTL-L2 cell line (Fig. 2). The equi-