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medsci@tubitak.gov.tr

Production and Purification of Monoclonal Antibodies to Human Interleukin-2 and Their Use for Immunoaffinity Purification of Recombinant Human Interleukin-2

Cemalettin AYBAY
Department of Immunology, Faculty of Medicine, Gazi University,
06500, Besevler, Ankara, Turkey

Abstract: Monoclonal antibodies (mAbs) are a powerful immunochemical tool. Today, the availability of mAbs with desired specificity enables ligand separation based upon the immunoaffinity technique. By using the high selectivity of mAbs, immunoaffinity purification offers the possibility of isolating compounds even from complex samples with a selectivity which cannot be achieved by other methods. This paper reports the preparation and identification of monoclonal antibodies against human interleukin-2 (IL-2) and the establishment of an immunoaffinity method for purifying human IL-2 (hIL-2). Hybridomas were generated by the fusion of NSO or FO myelomas and spleen cells from immunized mice. Hypoxanthine-aminopterin-thymidine-resistant hybridomas secreting antibodies specific for hIL-2 were assayed by an ELISA and cloned using the single cell pick-up technique. The monoclonal antibody of the isotype IgM was purified from fetal calf serum (FCS)-free supernatant of hybridoma cell culture using ammonium sulfate precipitation followed by size-exclusion chromatography. The monoclonal antibody of the isotype IgG1 was purified using protein G affinity chromatography directly from supernatant of hybridomas cultured in medium supplemented with IgG-depleted FCS. In order to purify human IL-2 by immunoaffinity chromatography, anti-human IL-2 IgG1 mAb was coupled to Cyanogen Bromide-activated Sepharose 4B gel beads. Three monoclonal antibodies, designated CAy-IL2M, CAy-IL2Mb, and CAy-IL2G, have been produced against human interleukin-2. CAy-IL2M and CAy-IL2Mb were shown to be of the isotype IgM, and CAy-IL2G was shown to be of isotype IgG1. Hybridoma tissue culture supernatants were strongly positive by ELISA at dilution of up to 1/1000. Human recombinant IL-2 was purified by immunoaffinity chromatography on CAy-IL2G -Sepharose CL-4B (5x50 mm), with a recovery of nearly 80% at a high flow rate of 0.4ml per minute. In order to prevent substantial losses in the total antigenic activity, the elution step was effectively optimized. These results suggest that the anti-human IL-2 mAb established in this study is useful for one-step purification of recombinant human IL-2. These mAbs have great potential for the development of an immunoassay for measuring human IL-2 and their application in immunoaffinity chromatography could offer a valuable tool for the purification of natural human IL-2 secreted from mitogen plus lectin-stimulated peripheral blood mononuclear cells. Thus, mAbs directed against other epitopes that might be present on native IL-2 could be obtained in the future.

Key Words: Monoclonal antibody, human interleukin-2, immunoaffinity purification.

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