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论文

海洋硫酸多糖类药物聚甘古酯单克隆抗体的制备及其特性研究

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摘要:

目的制备和纯化聚甘古酯(911)单克隆抗体,为911药代动力学的免疫学方法的建立提供依据。方法 用还原胺化法,制备911-BSA和911-HSA复合物,间接ELISA法检测抗体生成。以IsostripTM试剂盒测定抗体亚型,流式细胞仪测定DNA含量。结果获得了一株阳性杂交瘤细胞DD3,腹水效价可达1×10⁵,其抗体亚型为IgG2a(κ),细胞株的DNA含量约为脾细胞和NS-1细胞之和,亲和力常数为2.0×10⁸ L·mol⁻¹。结论本实验制备了特异性针对911的单克隆抗体DD3,且与内源性多糖和褐藻酸无交叉反应,为911药代动力学的免疫学检测提供了依据。

关键词: 海洋硫酸多糖 聚甘古酯 单克隆抗体 抗艾滋病药物

Preparation and characterization of monoclonal antibody against marine sulfated polysaccharide drug poly-mannaguluronic acid

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Abstract:

Aim To prepare and characterize the monoclonal antibody against poly-mannaguluronic acid (911). Methods A hybridoma cell line was obtained by cloning for 3 times those cells that secret antibody against 911 after the fusion of NS-1 myelome cells with spleen cells from Balb/c mice immunized with a 911-bovine serum albumin conjugate, prepared by reductive amination. Hybridoma was inoculated to Balb/c mouse to induce ascites. The antibody was purified by ammonium sulfate and Protein A - Sepharose CL-4B. DD3 was confirmed to be fusion cells after determination of DNA content with flow cytometry. Competitive inhibitory test and Biacore confirmed the cross-reactivity of the antibody with other endogenous polysaccharides or with alginic acid sodium. Igs' classes and subclasses were identified by Isostrip $^{\rm TM}$ method. The affinity of DD3 was verified by ELISA. Results A hybridoma cell line secreting monoclonal antibody against 911 (marine sulfate polysaccharide) named DD3 was obtained. The DD3 ascites contained specific antibody with titer over $1.0 \times 10_5$. There was no cross-reactivity of these antibodies with other endogenous polysaccharides or with alginic acid sodium. The immunoglobulin subclass of DD3 was IgG2a, κ type. The affinity of DD3 was $2.0 \times 10_8$ L·mol $^{-1}$. Conclusion One hybirdoma line (DD3) secreting monoclonal antibody against 911 was established to provide a potential method for the pharmacokinetic study of 911.

Keywords: poly-mannaguluronic acid monoclonal antibody anti-AIDs agent marine sulfated polysaccharide

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