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日本七鳃鳗(*Lampetra japonica*)VLRB的克隆表达及单克隆抗体制备

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- 摘要
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摘要 七鳃鳗(*Lampetra japonica*)和盲鳗(*Hyperotreti*)作为现存的无颌类脊椎动物的代表, 其适应性免疫系统中的受体分子与哺乳动物的抗原受体分子不同, 这种独特的受体分子称为可变淋巴细胞受体VLRs(Variable lymphocyte receptors)。目前VLRs 分为3类, 分别是VLRA、VLRB、VLRC, 而VLRB由七鳃鳗类B淋巴细胞产生, 是其体液免疫中主要成分, 与IgM结构和功能类似。文章对日本七鳃鳗*VLRB*基因保守的C末端进行克隆、原核表达和重组蛋白纯化后, 免疫Balb/c小鼠, 通过细胞融合及间接酶联免疫吸附实验(Enzyme-linked immunosorbent assay, ELISA)筛选技术得到针对VLRB保守区的单克隆抗体细胞株。将杂交瘤细胞接种小鼠腹腔得到大量的单抗腹水, 经Protein G亲和纯化后的单抗进行ELISA与Western blotting检测。经ELISA检测抗体效价为 1:40000。Western blotting结果显示该单克隆抗体能够特异的检测重组VLRB蛋白及七鳃鳗血清中分泌型VLRB。流式细胞实验证明该单抗能特异识别七鳃鳗类淋巴细胞表面表达的膜型VLRB。VLRB单克隆抗体的成功制备和建株, 为研究日本七鳃鳗基于VLR的适应性免疫系统提供了重要的工具。

关键词： 七鳃鳗 可变淋巴细胞受体(VLR) VLRB 单克隆抗体

Abstract： The agnathans (lampreys and hagfishes) are representatives of the jawless vertebrates. The receptor molecules of adaptive immune system in lampreys are different from the antigen receptors in mammal vertebrates. The unique receptor molecules of lampreys are known as variable lymphocyte receptors (VLR). There are three types of VLRs in lampreys, VLRA, VLRB, and VLRC. Multimeric antigen-specific VLRB antibodies are secreted by VLRB+ lymphocytes and constitute the major components of the humoral arm of the lamprey adaptive immune system. Oligomeric VLRB antibodies are composed of four or five disulfide-linked dimeric subunits, which are similar to IgM antibodies in structure and function. In this study, the conservative c-terminal of *Lampetra japonica VLRB* was cloned and expressed in BL21 *E. coli*. The recombinant VLRB protein was purified by Ni^{2+} affinity chromatography column. After Balb/c mice immu-nity, cell fusion, the positive clones were screened by indirect enzyme-linked immunosorbent assay (ELISA). Finally, the hybridoma cells that produced specific anti-VLRB monoclonal antibodies were obtained. In order to get a large number of antibodies against VLRB, the hybridoma cells were injected into the abdominal cavity of Balb/c mice and the antibodies were purified by protein G sepharose. The results of ELISA indicated that the valence of anti-VLRB antibodies was 1:40000. Western blotting assay showed that the antibodies were able to detect both recombinant VLRB and secreted VLRB in lamprey sera. Flow cytometry analysis also revealed the existence of VLRB on the surface of lymphocytes. In summary, the anti-VLRB monoclonal antibodies provided a major tool for studying lamprey adaptive immune system.

Keywords： lamprey, variable lymphocyte receptor, VLRB, monoclonal antibody

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