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论著

重组小鼠Izumo的表达及其特异性抗体对小鼠体外精卵融合的影响

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摘要 背景与目的： 精子膜蛋白Izumo在精卵融合中起关键作用。本研究旨在表达和纯化重组小鼠Izumo蛋白(mIzumo)，探讨重组mIzumo在小鼠体内的免疫原性及其特异性抗体对小鼠体外精卵融合的影响。 材料与方法：将编码mIzumo蛋白的cDNA序列亚克隆到pET28a(＋)原核表达载体上，构建pET28a(＋)-mIzumo重组质粒。在BL21(DE3)大肠杆菌中表达重组6His-mIzumo融合蛋白，并通过Ni2＋亲和层析纯化。使用福氏佐剂乳化纯化的6His-mIzumo，分别免疫雌性和雄性C57BL/6小鼠，并采集免疫前和免疫后血清。用Western Blot和ELISA检测血清中抗6His-mIzumoIgG抗体的特异性和滴度。采用IVF和精卵融合试验检测血清中抗6His-mIzumo抗体对小鼠精卵融合的影响。 结果： 纯化的6His-mIzumo在SDS-PAGE凝胶上显示为约60 kD的单一条带。免疫了重组蛋白的雌性和雄性小鼠都产生了抗6His-mIzumoIgG抗体。抗6His-mIzumoIgG抗体与重组蛋白，小鼠睾丸、附睾及精子膜蛋白存在交叉反应，免疫印迹显示为约60kD的特异性条带。ELISA结果表明，免疫6His-mIzumo后至少6周之内，血清中的抗6His-mIzumoIgG抗体维持在最高水平。免疫后血清处理组的小鼠精子与去透明带卵母细胞发生胞膜融合的能力明显低于免疫前血清处理组(P<0.01)。 结论： 纯化的6His-mIzumo融合蛋白能够诱发雌性和雄性小鼠产生可阻断精卵融合发生的特异性血清抗体。因此，6His-mIzumo可作为同种异体抗原用于免疫避孕候选疫苗的研究。

关键词 Izumo；亲和层析； 精卵融合； 免疫血清 免疫避孕

Expression of Recombinant Mouse Izumo and Effect of Anti-mouse Izumo Antibody on Mouse Sperm-egg Fusion in vitro

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Abstract BACKGROUND & AIM: The sperm membrane protein Izumo was vital for sperm-egg fusion. The aim of this study was to express and purified recombinant mouse Izumo protein (mIzumo) and to assess the immunogenicity of the recombinant mIzumo and the effect of an antibody against recombinant mIzumo on mouse sperm-egg fusion in vitro. MATERIALS AND METHODS: The coding sequence of mIzumo was subcloned into pET28a(＋). The recombinant fusion protein, 6His-mIzumo, was expressed in E coli. BL21(DE3) strain and purified by Ni2＋ affinity chromatography. Mice of both sexes were immunized with purified 6His-mIzumo combined with Freund's adjuvant. Pre-immunized and post-immunized serum samples were tested for anti-6His-mIzumo activities by Western blot and ELISA. The effect of anti-6His-mIzumo antibodies on sperm-egg fusion was detected by in vitro fertilization (IVF) and sperm-egg fusion assay. RESULTS: The purified 6His-mIzumo appeared as a single ≈60 kD band on SDS-PAGE. Both immunized male and female mice developed serum antibodies, which, on Western blot, cross-reacted with a single ≈60 kD protein band corresponding to mIzumo in membrane protein extracts of mouse testis, epididymis and sperm. According to ELISA, the titre of anti-6His-mIzumo antibodies remained at the highest level for 6 weeks after final immunization. The ability of post-immunized sera treated sperm to fuse with zona-free oocytes was significantly inferior to that of the sperm treated with pre-immune sera (P<0.01). CONCLUSION: The purified 6His-mIzumo could stimulate immune response in mice of both sexes and induce specific antibodies which could inhibit sperm-egg fusion in vitro. Therefore, as an allogeneic antigen, 6His-

mIzumo could be as a candidate for development of immunocontraceptive vaccine.

Keywords Izumo affinity chromatography sperm-egg fusion immune serum immunocontraception

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