Transient Transfection with RNA and Modulation of
Human Lymphocytes by in vitro synthesized mRNA

OCR Number: OCR 4277

Description:

Efficient use of an in vitro produced chimeric mRNA and other modulator RNAs to create lymphocytes
with high levels of specific cytotoxicity against chosen targets.

Value Proposition: Our method can be used in developing cytotoxic T and NK lymphocytes,
macrophages, APC/dendritic cells and stem cells for adaptive therapy of various cancers and acute
infections.

Field of Application: Novel method to produce highly efficient cytotoxic T lymphocytes and NK cells in
safe conditions for potential patients.
1. Modulation of lymphocytes for cancer research and therapy.
2. Autoimmunity research and therapy.
3. Therapy of acute infections (viral and bacterial).
4. Stem cells research, including modulation of the developmental pathways.
5. Highly efficient lymphocyte modulation in basic immunology research

Advantages: Present technology of mRNA transfection is based on a method in which the gene of
interest has to be cloned in special plasmid vectors containing T7 promoter upstream and polyA/T
stretch downstream of the cloning site. This cloning procedure is time consuming and technically
complicated. The recombinant plasmids of that structure are often unstable and prone for spontaneous
mutagenesis. Our method does not require any cloning; it saves time and eliminates the problem of
plasmid instability. Our method can be used for delivery into cells and expression of multiple chimeric
immune receptors and other immuno-modulating genes. This method can even be applied on cells not
transfectable with DNA.

Our method is suitable for adaptive immunotherapy. It is more efficient than present technology
because it results in massive and fast accumulation of specific cytotoxic lymphocytes: more than 90%
of the lymphocytes uniformly expressed transgenic chimeric immuno-receptor (CIR) on their surface, as
well as other mRNA transgenes which can modulate lymphocyte viability and cytotoxic activity.

Stage of Development: We have demonstrated that human CTLs and NK cells can be transfected with
CIR mRNAs with high efficiency. After transfection, whole cell populations uniformly expressed chimeric
receptors. This method allows one to titer the expression level of transgenes, and multiple types of
mRNA can be simultaneously transfected. Human CTLs and NK cells loaded with CIR mRNA killed B
lymphoma cells, rhabdosarcoma, plasmocytoma, melanoma and colon carcinoma cells. The method
succesfully worked in vivo to markedly reduce tumor size in SCID/NOD mice bearing B-lymphoma
tumors. The overall time for lymphocyte preparation is relatively short, and determined by the time of
their collection or ex-vivo expansion. After a few hours of mRNA transfection, almost the entire
population of lymphocytes expressed the chimeric receptor and could be used in targeting experiments.

Publications:
Rabinovich PM et al., Human Gene Therapy, October 2006; 17(10):1027-35.

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