METHODS AND COMPOSITIONS FOR INCREASED TRANSGENE EXPRESSION

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[0001] Not applicable.

TECHNICAL FIELD

[0002] The present invention relates generally to improved methods and compositions for expressing exogenous nucleic acids in cells, and more particularly to improved expression of exogenous genetic material in human immune cells using adenoviral vectors.

BACKGROUND

[0003] Reliable transgene expression in immune effector cells such as T cells can provide needed insight into immunological mechanisms, and would also help enable novel immunotherapeutic strategies such as, e.g., gene therapy approaches for treating cancer as well as immunological, hematological, infectious, and genetic disorders. Unfortunately, however, stable and effective transgene expression in T cells has proven to be an~~bean~~ elusive goal, often requiring hit-or-miss experimentation. It is well-established, for example, that transduction of resting primary T lymphocytes with viral vectors, such as retroviral derived vectors, generally results in very low efficiencies [Yamashita and Emerman, J. Virology 2006 Jan 5;344(1):88-93]. Accordingly, studies in retroviral-mediated gene transfer to primary T lymphocytes over the past ten years have examined a number of different strategies to improve transduction efficiencies, leaving the even greater hurdle of satisfactory transgene expression as a more distant goal.

[0004] With respect to viral systems providing permanent integration into the host cell, it has been shown that T cell activation in conjunction with lentiviral transduction can significantly improve gene delivery to T cells. See, e.g., Unutmaz et al., J Exp Med. 1999 Jun 7;189(11):1735-46; Pollock et al., J. Virology, 1998 Jun;72(6):4882-92; Costello et al., Gene Ther. 2000 Apr;7(7):596-604; Bai et al., Gene Ther. 2003 Aug; 10(17):1446-57; Lu et al., J Gene Med. 2004 Sep;6(9):963-73. Bai et. al. and Lu et. al., for example, independently reported that both phytohemagluttinin (PHA) and anti-CD3/ antiCD28 costimulation of T lymphocytes can improve lentiviral gene delivery efficiencies, demonstrating 80 to >99%

transduction. Despite these reported successes in transduction efficiency, however, the actual levels of gene expression obtained by these researchers is inconclusive based on the reported data.

[0005] Unfortunately, however, attempts to reproduce these results using non-integrative viral systems have been more problematic. See, e.g., Schroers et al., Exp Hematol. 2004 Jun;32(6):53646. In the Schroers study, PHA activation provided only a modest improvement in transduction efficiency with adenoviral vectors, approaching only 45 percent transduction, significantly lower than that obtained using lentiviral vectors. Thus, there remains a significant need in the art for improved methods and compositions capable of high transgene expression in T cells in non-integrative viral systems, e.g., to provide proteins of therapeutic, industrial, or research uses. The present invention addresses this and other needs.

SUMMARY

[0006] The present invention derives from the surprising finding that a T cell co-stimulatory signal, unlike other forms of T cell activation, significantly improves adenoviral-mediated transduction of T cells with exogenous nucleic acids and, more importantly, produces high expression levels of an encoded exogenous molecule. The compositions and methods described herein allow for increased transient expression of any exogenous sequence in any T cell. Accordingly, the need for an integrating vector (retrovirus or lentivirus), which vectors can cause insertional mutagenesis or variegated expression due to position effects, is eliminated. In addition, the ability to transiently express exogenous sequences in T cells may provide for more stable cell populations, for example in that immunogenic peptides may be cleared out before the host immune system can mount a response. Thus, as described herein, the exogenous molecule may be any diagnostic or a therapeutic molecule of interest, thus providing novel methods of diagnosing, treating and/or preventing a wide variety of conditions and disorders.

[0006a] In one aspect, there is provided a kit for increasing expression of a transgene encoding a polypeptide product in T cells, the kit comprising at least a first and second co-stimulatory agents, wherein the co-stimulatory agents are provided on one or more beads and further wherein the first co-stimulatory agent comprises a CD3 ligand; and an adenoviral vector comprising (i) a sequence encoding said transgene operably linked to a promoter and (ii) a sequence encoding at least one zinc finger nuclease.

[0006b] In another aspect, there is provided a pharmaceutical composition comprising at least first and second co-stimulatory agents, wherein the co-stimulatory agents are provided on one or more beads and further wherein the co-stimulatory agent comprises a CD3 ligand and the second co-stimulatory agent is a CD28 ligand or IL-15; and an adenovirus vector comprising (i) a sequence encoding a transgene operably linked to a promoter and (ii) a sequence encoding at least one zinc finger nuclease.

[0007] In one example~~aspect~~, methods for increasing expression of an exogenous nucleic acid in T cells are provided, comprising 1) activating a population of T cells in vitro or ex vivo with a co-stimulatory signal produced by at least a first and second co-stimulatory agent; and 2) contacting said activated T cell population with an adenoviral expression vector comprising said exogenous nucleic acid; wherein said contacting results in expression of a transgene encoded by the exogenous nucleic acid in greater than 50% of said activated T

cells. In one example~~embodiment~~, the T cell is a CD4+ T cell. In another example~~embodiment~~, the T cell is a CD8+ T cell.

[0008] In one example~~a preferred embodiment~~, the co-stimulatory signal includes activation of the T cell receptor, and employs a co-stimulatory agent comprising a CD3 ligand. In another example~~a particularly preferred embodiment~~, the CD3 ligand comprises an anti-CD3 antibody. In certain example~~embodiments~~, expression of the endogenous gene is at least about three fold greater, preferably at least about five fold greater and even more preferably at least about six fold greater as compared to T cell activated with PHA and/or IL 2 and transduced with an adenoviral vector.

[0009] In one example~~a preferred embodiment~~, the co-stimulatory signal also includes activation of the CD28 receptor, and the co-stimulatory agent further comprises a CD28 ligand. In another example~~a particularly preferred embodiment~~ the CD28 ligand comprises a CD28 antibody.

[0010] In yet another example~~an alternative embodiment~~, the co-stimulatory agent further comprises IL-15. In a further example~~another alternative embodiment~~, the co-stimulatory signal further comprises a feeder cell.

[0011] In one example~~a preferred embodiment~~, the adenoviral expression vector is pseudotyped to enhance targeting of immune cells, e.g., T cells. In one example~~embodiment~~, the pseudotyped adenovirus expression vector comprises sequences from Ad5 and Ad35 adenoviruses. In one example~~a particular embodiment~~, the Ad35 sequence is F35. In another example~~embodiment~~, the pseudotyped adenovirus expression vector comprises sequences from Ad5 and Ad 11 adenoviruses.

[0012] In one example~~aspect~~, the exogenous nucleic acid encodes a diagnostic molecule such as, e.g. a fluorescent protein (*e.g.*, GFP or RFP).

[0013] In another example~~aspect~~, the exogenous nucleic acid encodes a therapeutic molecule such as, e.g., an RNA molecule or a therapeutic protein. In certain examples~~preferred embodiments~~, the therapeutic protein is selected from the group consisting of hormones, enzymes, cytokines, chemokines, antibodies, mitogenic factors, growth factors, differentiation factors, factors influencing angiogenesis, factors influencing cell apoptosis, and factors influencing inflammation.

[0014] In another example~~aspect~~, the exogenous nucleic acid encodes for a molecule capable of disrupting, enhancing or altering endogenous gene expression such as, e.g., transcription factors or nucleases. In certain examples~~preferred embodiments~~, the exogenous nucleic acid encodes for a zinc finger protein. In one example~~a particular embodiment~~, the zinc finger protein is directed to CCR5.

[0015] In another example~~aspect~~, the exogenous nucleic acid sequence may produce one or more non-coding sequences, for example, one or more RNA molecules (e.g., small hairpin

RNAs (shRNAs), small interfering RNAs (siRNAs), inhibitory RNAs (RNAis), microRNAs (miRNAs), etc.).

[0016] In one exampleaspect, the disclosureinvention provides an improved method of integrating an exogenous sequence into a T cell, said method comprising: a) activating said T cell with a co-stimulatory signal; and exposing said activated T cell to an adenoviral vector comprising said exogenous sequence, for example, using ZFN-mediated targeted integration. In certain examplembodiments, the integration of the exogenous sequence is at least about 70% to 90% efficient, including any value therebetween (*e.g.*, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%).

[0017] In one exampleaspect, kits for expressing and/or integrating an exogenous sequence in T cells are provided comprising a costimulatory agent as described herein and an adenoviral expression vector.

[0018] In one exampleaspect, methods of cleaving an endogenous gene in a cell are provided comprising expressing at least one zinc finger nuclease in a T cell according to any of the methods described herein, such that the zinc finger nuclease(s) cleave(s) the endogenous gene. In certain examplembodiments, the zinc finger nuclease cleaves a gene encoding a receptor involved in HIV entry into a cell (e.g., CCR5). In other examplembodiments, the zinc finger nuclease(s) cleave(s) a glucocorticoid receptor (GR). Thus, also provided are methods of preventing and/or treating HIV by expressing a CCR-5 binding zinc finger nuclease in a T cell. Methods of retaining T cell immune function during glucocorticoid treatment by cleaving a GR are also provided.

[0019] Additional features and advantages of the invention will be set forth in the detailed description which follows, and in part will be readily apparent to those skilled in the art from that description or recognized by practicing the invention as described herein, including the detailed description which follows, the claims, as well as the appended figures.

[0020] It is to be understood that both the foregoing general description and the following detailed description present embodiments of the invention, and are intended to provide an overview or framework for understanding the nature and character of the invention as it is claimed. The accompanying drawings are included to provide a further understanding of the invention, and are incorporated into and constitute a part of this specification. The figures illustrate various embodiments of the invention, and together with the description serve to explain the principles and operations of the invention.