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REVIEW

Macrophage Gene Therapy: opening novel therapeutic avenues for immune disorders [version 1; peer review: 1 approved with reservations, 1 not approved]

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Abstract

Macrophages are probably the most important cells of the mammalian immune system, and compromised macrophage function is known to cause several diseases. Their involvement in arthritis, cancer, infections, atherosclerosis, diabetes, and autoimmune disorders is well known. There has been a constantly growing need to transfer therapeutic genes into macrophages. Like most non-macrophage gene therapies, *in vitro* gene transfer has been attempted much more frequently in case of macrophages. However, primary macrophages are still somewhat recalcitrant to transfection. Macrophage-specific synthetic promoters, which were recently used successfully, can have up to 100-fold higher activity than that of native promoters. Adenovirus, lentivirus, and adeno-associated virus are commonly used for macrophage gene therapy. A number of non-viral methods are also popular for the transfer of exogenous DNA into macrophages. Gene transfer to macrophages using naked DNA has also been successful in a few cases. Macrophages have specific mechanisms to recognize and respond to bacterial DNA because of the presence of unmethylated CpG dinucleotides, which are rare in eukaryotic DNA. With interesting developments in this area, macrophage gene therapy appears to have great potential for immune therapies.

Keywords

Macrophage , Gene Therapy , Immunotherapy

Open Peer Review

Approval Status

	1	2
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1. Natalia Iago , Institut Pasteur de Montevideo, Montevideo, Uruguay		
2. Zhuling Guo , University of Hong Kong, Pok Fu Lam, Hong Kong		

Any reports and responses or comments on the article can be found at the end of the article.

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Introduction

Macrophages are known to be involved in the development of various diseases, not only because of their key role in host immunity, but also because of their ability to act as a host and reservoir for certain pathogens^{1,2}. Their involvement in rheumatoid arthritis, tumorigenesis, AIDS, atherosclerosis, diabetes, and lupus erythematosus has already been well documented (3–6; Figure 1). Since, like other primary cells, primary macrophages also resist transfection by contemporary methods, efficient transfection methods are required⁷. A recent breakthrough in macrophage gene therapy is the successful use of macrophage-specific synthetic promoters. These promoters were made by random combination of macrophage and myeloid cis elements⁸. This promoter was found to have up to 100-fold higher activity than that of a native macrophage promoter with minimal activity in nonmyeloid cells. When this promoter was used with GFP, blood leukocytes showed stable and high levels of CD11b+ specific GFP expression for more than a year. Similarly, hypoxia-response elements (HREs) are being utilized for delivery of therapeutic genes specifically to hypoxic tumour areas⁹. Self-inactivating retroviral vectors that had regulatory cis elements from the macrophage-specific human *CD68* gene, successfully directed

cell-specific expression of reporter genes in immune cells *in vivo*¹⁰. Furthermore, a number of gene therapy trials, where macrophage granulocyte-macrophage colony-stimulating factor (GM-CSF) expression was altered, showed promising results¹¹.

Viral gene therapy

Viruses including adenovirus, lentivirus, and adeno-associated virus are commonly used to transfect macrophages¹². However, a number of non-viral methods have also been used¹³. The biggest disadvantage of non-viral methods is that exogenous DNA entering the macrophage cells by endocytosis is often degraded by nucleases. On the other hand, viral methods generally give higher transfection efficiency and sustained transgene expression¹⁴. Retroviruses (other than the lentivirus sub-family) are generally ineffective in the transfection of primary macrophages as these cells are of non-dividing nature¹⁵. Therefore, macrophages are often generated from retrovirus-infected macrophage precursors that divide more frequently¹⁶. Convenient transfection of macrophage precursors offers a greater advantage of re-introduction of transfected cells into a suitable host. An encouraging macrophage-infection rate of 90% was seen, when a nuclear localization signal (NLS) sequence was introduced into

Figure 1. Prospects of macrophage gene therapy.

the matrix protein of the C type retrovirus¹⁷. A common problem with retroviruses is that transgene expression from integrated retroviruses is lost after a short duration, possibly due to transcriptional silencing.

Lentiviruses can normally infect non-dividing cells including monocytes. Viral matrix proteins, Vpr, integrase, and *pol* genes have been shown to be important for the transduction of non-dividing cells in this case¹⁸. Lentiviral vectors might also be less susceptible to transcriptional silencing. Adenoviruses can infect non-dividing cells with high efficiency but with moderate stability. 10–80% transfection efficiencies of human macrophages have been reported using adenoviruses¹⁹. Interestingly, adenoviruses are less capable of infecting monocytes. However, more than 90% infection rate was reported, where primary human monocytes were pre-treated with macrophage-colony stimulating factor⁸. Despite a number of advantages, the major drawback of lentiviral vectors (particularly of human lentiviruses) is the extreme safety concerns over its use⁸. Adeno-associated viruses (AAV) offer some advantages similar to retroviruses; they can insert themselves into the host cell chromosome for stable expression of transgenes, and, they can also infect both proliferating and non-proliferating cells²⁰. One of the common drawbacks of using AAV or retroviral vectors for gene therapy is that they offer low size limits (4–8 kb). On the other hand, poxvirus and herpes simplex virus, that have also been used to infect macrophages, have much higher capacity. A major hurdle in viral gene therapy is immune responses generated by viral vectors especially in the case of adenoviruses and AAV. The common solution for these problems is to use a viral vector having a truncated genome and therefore produce fewer viral proteins that can be immunogenic^{21,22}. Importantly, most of the viruses used so far infect a broad range of cell types, and are not highly specific for macrophages. Altering the cell tropism of viruses to enhance specificity has been an important area of research recently²³. Some of the interesting reports, where an artificial virus cell-binding receptor was engineered, have shown promising results²⁴. Also, genetic manipulation of the genes coding for the adenoviral fiber protein was helpful to gain higher specificity.

Non-viral gene therapy

Both chemical and physical methods including liposomes, lipoplexes, DNA carriers, diethylaminoethyl (DEAE)-dextran, microinjection, and electroporation have been used with variable success rates²⁵. Some of the drawbacks of non-viral methods are low transfection efficiency and transient transgene expression. The other common problems are: limited systemic application, rapid clearing from the circulation, and transfection of non-target tissues^{26–28}. This can be a problem during gene therapy. Fortunately, this problem can easily be avoided by treating the transfection-ready DNA with a methylase. Although electroporation has been shown to achieve moderate levels of transfection efficiency for human monocytic cell lines

and monocyte-derived macrophages, the main disadvantage in this case is increased cell death²⁹. Improved transfection reagents offer benefits like low toxicity, large DNA delivery, and lack of immunogenicity³⁰. However, poor transfection efficiencies were seen in the case of primary macrophages¹⁹. LipofectAMINE along with protamine sulfate was found to be the most effective in macrophage cell line RAW 264.7, followed by Lipofectin, DOTAP, and DEAE-dextran³¹. Cationic liposome/DNA complexes have also been used for transfecting monocytes/macrophages *in vivo* in the blood, liver, and spleen³². However, a number of non-target cells were transfected in these cases. Several transfection methods have been developed to target specific macrophage cell surface receptors³³. Ligands such as mannose and transferrin have been incorporated into gene transfer vehicles to increase the efficacy of transfection for macrophages. Some of the intracellular microbes that infect macrophage cells were also used as a method of transferring DNA constructs. An attenuated derivative of *Salmonella typhimurium* containing interferon-expressing plasmid has been used for macrophage-specific interferon expression³⁴. Similarly, attenuated mutants of leishmania have been developed that can safely be used for macrophage gene therapy^{35,36}.

Conclusion

Macrophages are of central importance in immune system, and also have important physiological roles in major organs including brain and liver. Their role in variety of immune, physiological, and pathogen-centric disorders have been repetitively validated. More and more diseases are showing associations with compromised or exaggerated macrophage functions. With the recent developments in the gene therapy, macrophages have become an attractive target to prevent or cure major diseases including cancer, range of blood-vascular disorders, diabetes, and various immune disorders.

Author contributions

GS and PB conceived the study and prepared the first draft of the manuscript.

UCP and CC did the analysis of the literature. PS did cross-checking and referencing.

Competing interests

No competing interests were disclosed.

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Zhuling Guo

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This article deals with a novel approach to immune disorders via the macrophage gene therapy. I personally think that it has reached to the threshold level of a short review. It is relatively easy to gain the main points that the authors try to summarize here. Therefore, a brief comment attached for further modification.

It is better to elaborate in-depth to support the current topic, or at least make your focal claims stand out. Another concern is that the authors are expected to point out some experimental directions or perspectives in this field to facilitate the next original works. Lastly, there are numerous abbreviations should be presented or listed throughout the text.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 22 October 2015

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Natalia Iago

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The present article attempts to review a very important topic in the immunology and gene therapy fields regarding the available tools to target and modulate macrophages using gene therapy. The field of gene therapy has evolved tremendously in the last 10-15 years, but the attempt to target macrophage still remains challenging. However, this reviewer has some major concerns about the article:

- A review should conform a critical view of the classical information and the latest developments, carefully cited to acknowledge the original authors of the experiments, and importantly, provide with a deep analysis and sum-ups with the personal interpretation or insights of the authors. It should suggest the future directions in the field and possible experiments or models to be used for a significant further advance. This review does not fulfill these requirements.
- The presented text is not well organized in a focused vision, which makes it difficult to go through. Moreover, the text suffers from a lack of deep insights in the gene therapy field.
- Carefully designed figures and tables should be presented to contribute to the establishment of a central idea of the article. For instance, the reviewer feels that a table summarizing the viral and non-viral vectors that have been used for targeting macrophages and their corresponding references would help to a better understanding of the article. Moreover, the only figure present in this review does not involve gene therapy and has no figure legend.
- Authors should be careful with the bibliography. There seems to be a mismatch between the citations in the text and the list of references.
- Finally, and most importantly, the reviewer feels that authors do not have experience in this field to be able to write this type of review.

In conclusion, the reviewer does not approve this article.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

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