Approaches to enhancing the retroviral transduction of human synoviocytes

ABSTRACT

This report concerns a clinical trial for rheumatoid arthritis (RA), approved by the US National Institutes of Health and the Food and Drug Administration. An amphotropic retrovirus (MFG-IRAP) was used ex vivo to transfer a cDNA encoding human interleukin-1 receptor antagonist (IL-1Ra) to synovium. The protocol required the transduced cells to secrete at least 30 ng IL-1Ra/106 cells per 48 h before reimplantation. Here we have evaluated various protocols for their efficiency in transducing cultures of human rheumatoid synoviocytes. The most reliably efficient methods used high titer retrovirus (approximately 108 infectious particles/ml). Transduction efficiency was increased further by exposing the cells to virus under flow-through conditions. The use of dioctadecylamidoglycylspermine (DOGS) as a polycation instead of Polybrene (hexadimethrine bromide) provided an additional small increment in efficiency. Under normal conditions of static transduction, standard titer, clinical grade retrovirus (approximately 5 × 105 infectious particles/ml) failed to achieve the expression levels required by the clinical trial. However, the shortfall could be remedied by increasing the time of transduction under static conditions, transducing under flow-through conditions, or transducing during centrifugation.

INTRODUCTION

Introduction Rheumatoid arthritis (RA) is a promising new target for gene therapy (reviewed in). One approach to the genetic therapy of RA requires the transfer of anti-arthritis genes to the synovial linings of joints. The first human trial of arthritis gene therapy approved by the Recombinant DNA Advisory Committee (RAC) of the National Institutes of Health and by the US Food and Drug Administration (FDA) involves the ex vivo, retroviral delivery to joints of a cDNA encoding the human interleukin-1 receptor antagonist (IL-1Ra). The procedure has been shown to be safe and effective in several animal models of RA. When rabbit type B synoviocytes are transduced with the amphotropic retrovirus MFG-IRAP under standard, static conditions, they routinely secrete approximately 100 ng human IL-1Ra/106 cells per 48 h into their culture medium. For the purposes of the clinical trial, transduced human synoviocytes were required to secrete at least 30 ng IL-1Ra/106 cells per 48 h. We investigated several transduction strategies in order to identify conditions that result in the highest transduction efficiency for use in this clinical trial. This communication describes attempts to improve the transduction efficiency of human rheumatoid synoviocytes by MFG-IRAP.

CONCLUSION

Conclusions Collectively, these data suggest that, of the parameters evaluated here, the single biggest improvement in retroviral transduction of human synoviocytes is obtained with high titer retrovirus. Further increases in transgene expression were achieved by using high titer retrovirus with flow-through transduction or centrifuging. When these factors were combined, IL-1Ra production was increased 50- to 100-fold relative to static transductions performed with standard titer virus. Improvements of this magnitude will be particularly important when performing gene therapy with transgenes such as IL-1Ra, which need to be produced in large molar excess over the molecules whose activities they antagonize.