Development of stealth transgenes for gene therapy : evaluation of cis-acting inhibitors of antigen presentation

Raamsman-Ossevoort, M.

Chapter 1

Introduction

Part 1

Aim and outline of this thesis

Part I

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The aim of the studies presented in this thesis is to develop and evaluate a technique to make transgene products used in gene-therapy applications "invisible" (i.e. stealthed) to the immune system. In addition to the use of previously described Gly-Ala repeat (GAr) domain of the Epstein-Barr virus nuclear antigen-1 (EBNA-1) we identified and studied new inhibitors, from EBNA-1 itself, and from another herpes-virus protein.

In **Chapter 1**, **part 2.1**, we give a general overview of gene therapy and cancer gene therapy. We briefly discuss the status of the clinical studies, and the vectors and genes used in these studies. One of the hurdles in gene therapy is the immune system. In **part 1.2.2** we discuss immune responses against one of the most frequently used vectors for gene therapy, the adenoviral vector. In addition, we evaluate the solutions that have been proposed as well as their feasibility. It is not only an immune response to the (viral) vectors that hampers the applicability of them in gene therapy. There is also ample evidence of transgene-product induced immune response. **Part 1.2.3** reviews this problem. It is evident that many viruses have evolved strategies to counteract the presentation of *neo*antigens. Some of their mechanisms are reviewed in part **1.2.4**.

The cellular immune response against transgene-encoded *neo*antigens is a major hurdle in gene therapy applications where long-term expression of transgenes is desired. Therefore new approaches are needed to prevent rapid clearance of transduced cells. We exploited the Gly-Ala repeat (GAr) domain of the EBNA-1, since the GAr prevents cytotoxic T lymphocyte epitope generation. In **Chapter 2**, the first results on this domain are described. Our data show that the fusion proteins retain their activity and we show how the GAr can be used to stealth transgene products.

Upon closer examination of the EBNA-1 gene we found a nested ORF. In this ORF there was a long repeat present, but because of the frame shift, this consisted of the acidic residues glutamine, glutamic acid, and glycine. We therefore named this repeat the GZr. We tested this repeat in the same way as the GAr and could show that also this repeat is capable of inhibiting antigen presentation in vitro. The results are described in **Chapter 3**.

The herpes simplex virus 1 (HSV-1) thymidine kinase (TK) is frequently used as a produg-activating enzyme in experimental gene therapy. However, in some studies a cellular immune response was mounted against this enzyme, thereby thwarting the therapy. **Chapter 4** deals with the modifications we made in the HSV-TK gene to blunt the immune response. First we fused HSV-TK with the GAr. In addition, we introduced modifications, which would

prevent splicing-out of the codons coding for the active site, and we made point mutations that have been described to enhance the affinity for the prodrug gancoclovir (GCV).

Since the GAr works very efficient in preventing a harmful immune response we set out to identify other proteins with similar functions. The kaposi sarcoma herpes virus (KSHV) a.k.a. human herpes virus 8 (HHV-8) has a protein that is, like EBNA-1, involved in episomal maintenance of the virus genome. This protein, the latency-associated nuclear antigen-1 (LANA-1), has a long acidic repeat. Remarkably, the last one-third of the repeat is strongly similar to newly found GZ-repeat protein that is encoded by the EBNA-1 ORF. In **Chapter 5** we show that also LANA-1 can affect the presentation of antigens.