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

Raamsman-Ossevoort, M.

Chapter 6

General Discussion

General Discussion

The studies presented in this thesis aimed at developing and evaluating a new technique to make transgene products used in gene-therapy applications "invisible" (i.e. stealthed) to the immune system. To this end we used the Gly-Ala repeat (GAr) domain of the Epstein-Barr virus nuclear antigen-1 (EBNA-1). We provided four examples of fusions with the GAr that did not impede the function of the reporter, i.e. the *E.coli*-derived β-galactosidase, herpes simplex virus-derived thymidine kinase (HSV-TK), firefly luciferase, and jellyfish green fluorescent protein (GFP).

We showed that the GAr does not affect induction of transgene directed CTL activity upon adenovirus-mediated transfer and expression of a GAr-LacZ fusion gene. However, recognition of transgene-expressing cells by antigen-specific CTL is abolished sufficiently to prevent cytolysis by β-galactosidase-specific CTL. The observation that the GAr does not inhibit priming of CTL is important and consistent with reports describing the presence of EBNA-1 specific-CTL in EBV-seropositive individuals (Blake et al., 1997; Blake et al., 2000; Rickinson and Moss, 1997). This inability of completely preventing the generation and presentation of antigenic peptides, may be attributed to the fact that the GAr seems not entirely prevent formation of defective ribosomal products (DRiPs) (Voo et al., 2004; Tellam et al., 2004; Lee et al., 2004). These DRiPs play an important role in antigenic peptide generation (Yewdell et al., 1996; Schubert et al., 2000; Reits et al., 2000) and are actually the main source of antigenic peptides for long-lived proteins (Khan et al., 2001). However, there are marked differences between our studies and the DRiPs study of Tellam and collaborators. They studied the full length EBNA-1 in human B-cells and lymphoblastoid cell lines (LCLs). Whereas specific lysis is observed in the GAr-containing EBNA-1, more efficient lysis could be obtained when the GAr was deleted from EBNA-1, confirming a stealthing effect of the GAr in the natural context. This is in accordance with the data from Lee et al., who also noted a partial protective effect of GAr in its natural context. Furthermore, deletion of the GAr greatly reduces the half-life of EBNA-1 in B cells. This is consistent with a negative effect of the GAr on the efficiency of proteasomal degradation (Levitskaya et al., 1995). In addition, we have studied the effect of the GAr with rather stable proteins and therefore they may require less stabilization by the GAr, to sufficiently inhibit antigenic-peptide formation.

We showed that the GAr does not affect induction of transgene directed CTL activity upon adenovirus-mediated transfer and expression of a GAr-LacZ fusion gene. As antigen presentation by professional APC, most likely DC, is crucial to the initiation of virus-specific CTL responses, the presence of EBNA-1-specific CTL in EBV-positive donors suggests that antigen processing for MHC class I by specialized APC is not hampered by the GAr. Our data strongly suggest that cross-priming does occur, since we could show priming in an indirect

manner (non-matching MHC class I molecules). The presence of this EBNA-1-specific CTLs points to the possibility that GAr does not affect the processing of exogenously acquired EBNA-1 antigens. This can be explained by the fact that in the cross-priming pathway upon phagocytosis of exogenous proteins there is limited proteolysis and these degradation products are exported to the cytosol (Lizee et al., 2003; Guermonprez et al., 2003; Houde et al., 2003; Ackerman et al., 2003). This limited proteolysis probably results in the separation of the GAr from the antigenic CTL epitope. In this way, the processing of the CTL epitope is liberated from the inhibitory influence of the GAr on proteasomal antigen degradation, explaining the observation that CTL priming proceeds in an uninhibited fashion.

In addition to the GAr, we showed that an alternative ORF in EBNA-1 can also inhibit presentation of linked antigens. This ORF contains a repeat of the same length as GAr and is rich in Gly (G), Glu and Gln (Z) residues. Therefore we named it the GZ-rich repeat. Remarkably, this repeat is strongly similar to the last one-third of the latency-associated nuclear antigen-1 (LANA-1) from kaposi sarcoma herpes virus (KSHV) a.k.a. human herpes virus 8 (HHV-8). This protein is, like EBNA-1, involved in episomal maintenance of the virus genome. Also this repeat region is implicated with inhibition of antigenic presentation. This together suggests that (long) repeats in general have an inhibitory effect on antigen presentation. It is speculated by Sharipo and collaborators (2001) that the inhibitory effect of the GAr requires at least three alanine residues in a β-strand conformation with adjacent hydrophobic binding pockets of a putative receptor (Sharipo et al., 2001). In the case of the GZr and the LANA-1, there are no alanine residues present and since the whole repeat is strongly acidic, it is highly unlikely that there will be hydrophobic pockets involved in the receptor part. This suggests that the GZr and LANA-1 repeats may function in a different way.

In addition to the full-length repeats of GAr and GZr we were able to obtain mini-repeats. These mini-repeats were tested as well since it is known that short EBNA-1 derived glycine and alanine-rich sequence of only 8 amino acids are able to significantly inhibit proteasomal degradation of instable reporter protein (Sharipo et al., 2001; Sharipo et al., 1998; Dantuma et al., 2000). Surprisingly, the mini-repeats blocked presentation more efficiently than the full length repeats. The mechanism for the more potent inhibition is unclear. Maybe these shorter proteins form less DRiPs then longer proteins, on the basis of size and inherent difficulties in folding or assembly (Schubert et al., 2000).

Together, these results make out a small library of inhibitory sequences for the generation of “immunostealth” genes. This is useful in gene therapy approaches where a life-long restoration of the defects is required. Furthermore, it can be used to create safer suicide genes from bacterial or viral origin. The most widely used suicide gene is the HSV-TK. Since this is a viral gene, problems can occur with its use. Indeed, upon infusion of HSV-TK-expressing T lymphocytes, 8 of 24 recipients developed an immune response against the transgene product (Thomis et al., 2001). To circumvent this problem attempts to use human

genes as suicide system, have been done. Of course in the use of human genes for suicide gene therapy, as is the case with Fas (Thomis et al., 2001) or caspase 8 (Carlotti et al., 2005) stealthing may not be necessary. However, the TK and ganciclovir combination has a very useful bystander effect, which makes this combination the system of choice for many gene therapy applications.

References

Ackerman, A. L., Kyritsis, C., Tampe, R., and Cresswell, P. (2003). Early phagosomes in dendritic cells form a cellular compartment sufficient for cross presentation of exogenous antigens. *Proc.Natl.Acad.Sci.U.S.A* **100**, 12889-12894.

Blake, N., Haigh, T., Shaka'a, G., Croom-Carter, D., and Rickinson, A. (2000). The importance of exogenous antigen in priming the human CD8+ T cell response: lessons from the EBV nuclear antigen EBNA1. *J.Immunol.* **165**, 7078-7087.

Blake, N., Lee, S., Redchenko, I., Thomas, W., Steven, N., Leese, A., Steigerwald-Mullen, P., Kurilla, M. G., Frappier, L., and Rickinson, A. (1997). Human CD8+ T cell responses to EBV EBNA1: HLA class I presentation of the (Gly-Ala)-containing protein requires exogenous processing. *Immunity.* **7**, 791-802.

Carlotti, F., Zaldumbide, A., Martin, P., Boulukos, K. E., Hoeben, R. C., and Pognonec, P. (2005). Development of an inducible suicide gene system based on human caspase 8. *Cancer Gene Ther.* **12**, 627-639.

Dantuma, N. P., Heessen, S., Lindsten, K., Jellne, M., and Masucci, M. G. (2000). Inhibition of proteasomal degradation by the gly-Ala repeat of Epstein- Barr virus is influenced by the length of the repeat and the strength of the degradation signal. *Proc.Natl.Acad.Sci.U.S.A* **97**, 8381-8385.

Guermonprez, P., Saveanu, L., Kleijmeer, M., Davoust, J., Van Endert, P., and Amigorena, S. (2003). ER-phagosome fusion defines an MHC class I cross-presentation compartment in dendritic cells. *Nature* **425**, 397-402.

Houde, M., Bertholet, S., Gagnon, E., Brunet, S., Goyette, G., Laplante, A., Princiotta, M. F., Thibault, P., Sacks, D., and Desjardins, M. (2003). Phagosomes are competent organelles for antigen cross-presentation. *Nature* **425**, 402-406.

Khan, S., de Giuli, R., Schmidtke, G., Bruns, M., Buchmeier, M., van den, B. M., and Groettrup, M. (2001). Cutting edge: neosynthesis is required for the presentation of a T cell epitope from a long-lived viral protein. *J.Immunol.* **167**, 4801-4804.

Lee, S. P., Brooks, J. M., Al Jarrah, H., Thomas, W. A., Haigh, T. A., Taylor, G. S., Humme, S., Schepers, A., Hammerschmidt, W., Yates, J. L., Rickinson, A. B., and Blake, N. W. (2004). CD8 T cell recognition of endogenously expressed epstein-barr virus nuclear antigen 1. *J.Exp.Med.* **199**, 1409-1420.

Levitskaya, J., Coram, M., Levitsky, V., Imreh, S., Steigerwald-Mullen, P. M., Klein, G., Kurilla, M. G., and Masucci, M. G. (1995). Inhibition of antigen processing by the internal repeat region of the Epstein-Barr virus nuclear antigen-1. *Nature* **375**, 685-688.

Lizee, G., Basha, G., Tiong, J., Julien, J. P., Tian, M., Biron, K. E., and Jefferies, W. A. (2003). Control of dendritic cell cross-presentation by the major histocompatibility complex class I cytoplasmic domain. *Nat.Immunol.* **4**, 1065-1073.

Reits, E. A., Vos, J. C., Gromme, M., and Neefjes, J. (2000). The major substrates for TAP in vivo are derived from newly synthesized proteins. *Nature* **404**, 774-778.

Rickinson, A. B. and Moss, D. J. (1997). Human cytotoxic T lymphocyte responses to Epstein-Barr virus infection. *Annu.Rev.Immunol.* **15**, 405-431.

Schubert, U., Anton, L. C., Gibbs, J., Norbury, C. C., Yewdell, J. W., and Bennink, J. R. (2000). Rapid degradation of a large fraction of newly synthesized proteins by proteasomes. *Nature* **404**, 770-774.

Sharipo, A., Imreh, M., Leonchiks, A., Branden, C., and Masucci, M. G. (2001). cis-Inhibition of proteasomal degradation by viral repeats: impact of length and amino acid composition. *FEBS Lett.* **499**, 137-142.

Sharipo, A., Imreh, M., Leonchiks, A., Imreh, S., and Masucci, M. G. (1998). A minimal glycine-alanine repeat prevents the interaction of ubiquitinated I kappaB alpha with the proteasome: a new mechanism for selective inhibition of proteolysis. *Nat.Med.* **4**, 939-944.

Tellam, J., Connolly, G., Green, K. J., Miles, J. J., Moss, D. J., Burrows, S. R., and Khanna, R. (2004). Endogenous presentation of CD8+ T cell epitopes from Epstein-Barr virus-encoded nuclear antigen 1. *J.Exp.Med.* **199**, 1421-1431.

Thomis, D. C., Marktel, S., Bonini, C., Traversari, C., Gilman, M., Bordignon, C., and Clackson, T. (2001). A Fas-based suicide switch in human T cells for the treatment of graft-versus-host disease. *Blood* **97**, 1249-1257.

Voo, K. S., Fu, T., Wang, H. Y., Tellam, J., Heslop, H. E., Brenner, M. K., Rooney, C. M., and Wang, R. F. (2004). Evidence for the presentation of major histocompatibility complex class I-restricted Epstein-Barr virus nuclear antigen 1 peptides to CD8+ T lymphocytes. *J.Exp.Med.* **199**, 459-470.

Yewdell, J. W., Anton, L. C., and Bennink, J. R. (1996). Defective ribosomal products (DRiPs): a major source of antigenic peptides for MHC class I molecules? *J.Immunol.* **157**, 1823-1826.