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Foamy virus vectors

The usefulness of a perfect parasite

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Introduction

Recently, Bauer *et al*.[1] reported on the cure of a rare recessive disease by means of foamy virus (FV)-mediated gene transfer, which may help to revive the field of gene transfer to hematopoietic stem cells (HSCs) after some clinical drawbacks in previous years and a phase of new orientation that is not over yet and characterized by elaborated safety tests and the search for new vector systems.[2] It is the merit of David Russell in Seattle who has pushed the field of FV vector development into this pole position.

The disease

Leukocyte adhesion deficiency (LAD), the disease investigated in the study of Bauer *et al*., is a rare genetic disorder with a frequency of 1 in 10^6 live births.[3] Leukocytes from LAD patients are functionally disabled due to mutations in the gene coding for the common CD18 (or β_2) subunit of the four different β_2-integrins, that is, LFA-1 (CD18/CD11a), Mac-1 (CD18/CD11b), p15095 (CD18/CD11c) and CR4 (CD18/CD11d).[4] The surface antigen CD18, in complex with CD11, enables leukocytes to adhere to inflamed endothelium and migrate to sites of infections. In the case of LAD, leukocyte paresis results in lethal recurring bacteremias and abscesses, which are the clinical hallmarks of the disorder. Patients often exhibit persistent granulocytosis without pus formation, whereas their lymphocytes are unable to bind to C3bi opsonized bacteria.[5] Clinical severity is dependent on the CD18 expression levels with those patients having less than 1% of the normal CD18 levels being most affected, whereas patients with up to 10% of normal activity can make it to adulthood with medical care.[6] As LAD-affected children often lack a suitable bone marrow donor[7] and the bone marrow transplant procedure has a significant treatment-related mortality,[8,9] gene therapy approaches for such patients are fully justified.

The viral vector

Retroviruses are divided into two subfamilies: orthoretroviruses (OV) are distinguished from FV.[10] Gammaretrovirus (GV) and lentivirus (LV), the bases for most retroviral vectors in clinical use now, belong to the OV.[11] FV have the principal genetic order of LTR (long terminal repeat), gag–pol–env–accessory genes–LTR, reverse transcription and integration into the host cell genome in common with OV. However, a closer look will reveal differences in almost any aspect of replication.[12,13] Most prominent is the feature to reverse-transcribe the RNA (pre-) genome late in replication before the virus buds from the cell membrane.[14–16] The stability of the resulting virion DNA genome is believed to be one reason for the excellent capability of FV vectors to transduce rarely dividing cells, such as HSCs.[17] Other reasons are particular features of the viral envelope that mediate access to virtually any cell type of interest, although the viral receptor is still unknown.[18]

The FV vectors have evolved over the last 10 years to a level that makes them an attractive gene transfer vehicle for clinical applications. Some of their features will be briefly outlined below; for a more extensive review on these vectors, see reference Rethwilm A.[19]

The current vectors in use were originally derived from infectious molecular clones[20,21] of an FV isolate obtained from human material,[22] although later evidence for a chimpanzee origin was presented indicating a probable trans-species transmission.[23] FV vectors have deletions in the U3 promoter region; deletions in most of their genome, with the exception of *cis*-acting sequences in *gag* and *pol*, required for genome packaging; and Pol protein encapsidation deletions in the transactivator and accessory genes. In effect, these vectors have room to accommodate about 9 kb of foreign DNA and are true SIN vectors as both the viral promoter and its transactivator are deleted.[24,25] FV vectors are produced in 293T cells by transient transfection of vector along with three different helper plasmids coding for *gag*, *pol* and *env*, thereby further minimizing the possibility of generating a replication-competent recombinant retrovirus. By concentration through centrifugation or filtration of the crude supernatant vector titers can be enhanced 100-fold without loss of infectivity.[26,27] However, vector titers and effective multiplicity of infection are less of an issue in FV applications, as comparable or even superior gene transfer rates can be obtained with a fraction of the multiplicity of infection as it has been shown in human and canine HSC transduction experiments, where FV vectors were tested head-to-head with GV and LV vectors.[28,29]

The integration pattern of FV vectors is also different from GV and LV;[30,31] compared with the former they have less preference to integrate into promoter close regions, and compared with the latter they have less preference to integrate into genes. Moreover, silencing of transgenes expressed from FV vectors has not been observed yet, indicating that FV vectors are suited for applications where long-term expression is a desired feature.[1,32–34] Recently, in another study addressing safety, Hendrie *et al*.[35] developed a plasmid-based assay to estimate the likelihood of an integrated retroviral vector to stimulate transcription of a nearby (indicator) gene with a minimal promoter, either by enhancer effects of transcriptional control elements in the vector backbone or by read-through from the long terminal repeat or internal promoter. Both phenomena are implicated in the activation of cellular proto-oncogenes by retroviral vectors.[2] Using this system, they compared GV- (MLV and SIN-MLV), LV- (HIV) and FV-derived vectors. Two of their findings are particularly worth mentioning here: (i) the choice

of the internal promoter has great influence on the propensity to activate the nearby gene in all vector systems analyzed and (ii) all assays revealed FV-derived vectors to be least likely to activate the nearby gene. Only time will prove if this assay, which relies on transient transfection of cell cultures, will be corroborated by *in vivo* studies. However, it is another argument in favor of FV vectors.

FV epidemiology

The FVs are probably the oldest exogenous retroviruses known; they have co-evolved with their hosts for the past approximately 60 million years.[36,37] In their natural hosts, they do not cause disease, and, importantly, are not associated with malignancies. 'Human' FVs are not known; however, transmissions of FVs from non-human primates to humans occur rather frequently, even in non-occupational settings. These trans-species transmissions give rise to persisting nonpathogenic infections and there is no evidence for the evolution of a genuine human FV.[38] There is also no evidence for human-to-human transmissions even by close contact, although only very few cases could be investigated.[39] It appears that FV can be regarded as perfect parasites that do not harm their hosts.

The canine LAD study

Bauer *et al*. studied a large animal model of LAD. There exists an Irish Setter breed with a CD18 gene mutation that perfectly mimics the situation in humans.[40] Despite antibiosis and intensive care, puppies that are homozygous for the gene defect usually die before reaching 6 months of age. Canine models are well accepted in the field of bone marrow transplantation and results from dog studies have been directly translated into human settings. After showing that FV vectors are well suited to transduce dog HSCs by a simple overnight exposure protocol, and that they are even superior to similar studies employing LV vectors,[28] the canine model of LAD was chosen to evaluate the usefulness of FV vectors in a 'real life' situation.

One year after transfusion, the percentage of gene-corrected leukocytes was on an average 3–4 times higher with the FV vector than in a previous study employing a GV vector.[41] Moreover, the analysis of integration sites revealed interesting differences between both vector systems: the FV vector did not show a preference greater than random to integrate in proximity of known oncogenes, whereas the GV vector displayed the established features.[1] Only marginal differences were found when the integration profiles into general transcriptional start regions were compared. However, what is probably most convincing is the sustained clinical benefit for the (animal) patients. The 'cured' dogs are by now approximately 3 years of age and do perfectly well without any signs of side effects due to the transplantation of the gene-corrected cells or silencing of transgene expression.

Efficacy issues

One reason that prevented a wider community to apply FV vectors was the relatively low titers obtained upon transient co-transfection of cells with vector and packaging plasmids. Now this hurdle has been overcome, and vector titers in excess of 10^7 per ml (as determined on human fibroblasts) are routinely produced in the labs of Myra O McClure (London), David W Russell (Seattle) and Helmut Hanenberg (Düsseldorf) without concentrating the vector particles (Personal communication). In addition, novel therapeutic FV vectors have been developed that can block HIV-1 replication,[42] whereas new vectors for the genetic correction of beta-thalassemia and chronic granulomatous disease have shown robust expression of the therapeutic protein and are under testing in the murine models (Vassilopoulos G, unpublished data). Together with the ease with which FV vectors transduce HSCs, this appears sufficient for a clinical application. Regarding this, it is likely that the FV envelope significantly contributes. Although it is not yet possible to efficiently pseudotype FV capsids with heterologous envelopes,[43] FV glycoproteins can be used to pseudotype OV particles. Very recently, Dirk Lindemann (Dresden) rescued an FV envelope mutant that allows HIV vector pseudotyping and transduction of human HSCs with 10 times the efficiency compared with the ubiquitously used VSV-G glycoprotein (Dirk Lindemann and Helmut Hanenberg, Personal communication).

Further implications

Accumulated evidence from marking experiments in murine, canine and human HSCs indicated that FV vectors could be an alternative system to the current GV- and LV-dominated gene therapy field. The canine LAD study verified the predictions for the therapeutic potential of the FV vectors and is an important milestone in the road toward a clinical study. Therapeutic FV vectors for other genetic disorders in murine preclinical models have been developed (Helmut Hanenberg, Personal communication; Vassilopoulos G, unpublished data) and will expand the repertoire of disorders amenable to FV-mediated HSC correction.

Research on FVs and FV vectors were pursued by only a handful of laboratories worldwide. Despite the interesting molecular aspects of replication, it was just their obvious *in vivo* nonpathogenicity that prevented funding agencies from supporting researchers to enter the field. This will hopefully change now that convincing studies that demonstrate the usefulness of this harmless parasite have been published. ■

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