Chapter 13

Lentiviral Vectors in Immunotherapy

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Additional information is available at the end of the chapter

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1. Introduction

Genetic immunotherapy can be defined as a therapeutic approach in which therapeutic genes are introduced into defined target cell types to modulate immune responses. A major challenge for this therapeutic strategy is the delivery of these genes into target cells in an efficient, stable manner. Possibly one of the best systems to achieve this is the use of lentiviral vectors (lentivectors) as gene carriers, as they are capable of transducing both dividing and resting cells [1].

Lentivectors are mainly derived from the human immunodeficiency virus (HIV-1) genome, a member of the *Retroviridae* family. The defining characteristic of retroviruses is their capacity to stably integrate their RNA genome into the host cell chromosomes, in the form of a cDNA copy (Figure 1). Therefore, retrovirus and lentivirus vectors have been used extensively in research since they are ideal gene carriers into target cells. Moreover, both retrovirus and lentivirus vectors have been successfully applied in human gene therapy for the treatment of several genetic/metabolic inherited diseases (Cartier et al, 2009; Cavazzana-Calvo et al, 2010; Gaspar et al, 2004; Grez et al, 2010; Ott et al, 2006; Thrasher et al, 2006).

Lentiviruses are spherical enveloped viruses with a diameter around 80 to 120 nm and contain two copies of a single-stranded RNA genome (Figure 2) [2]. The genome is enclosed within a core composed of the structural and enzymatic proteins nucleocapsid (NC), capsid (CA), reverse transcriptase (RT), integrase (IN) and protease (PR). The core is surrounded by a protein layer of matrix (MA) protein. The envelope protein (ENV) is embedded in the virion lipid envelope, and it binds to the target cellular receptor and mediates virion entry.

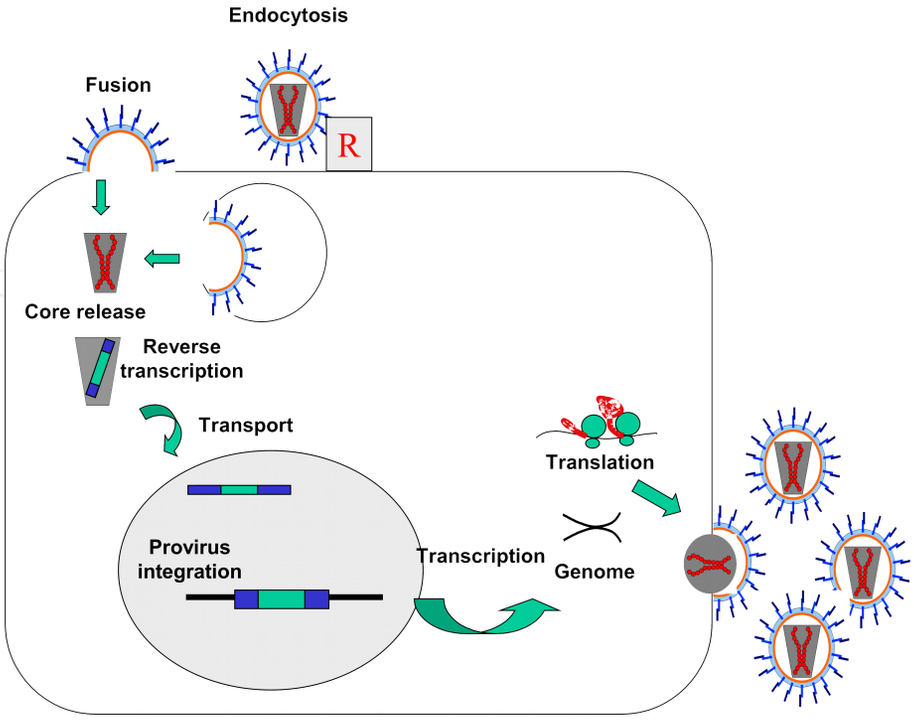

Figure 1. The retrovirus life cycle.The life cycle of retroviruses, including lentiviruses is shown in this figure as a multi-step mechanism, starting with virion binding to the cellular receptor (R), leading to direct fusion or endocytosis. Then, the internal core is released and the two RNA molecules undergo reverse transcription as indicated, ending up with a single cDNA molecule. The core is then transported to the nucleus (in the case of lentiviruses) and the cDNA is integrated into the cell chromosome. The integrated genome (provirus) undergoes transcription, producing more RNA genome copies (and also spliced mRNAs, not shown here), which are also translated into structural and enzymatic proteins. These are then assembled into virions that bud out of the infected cells.

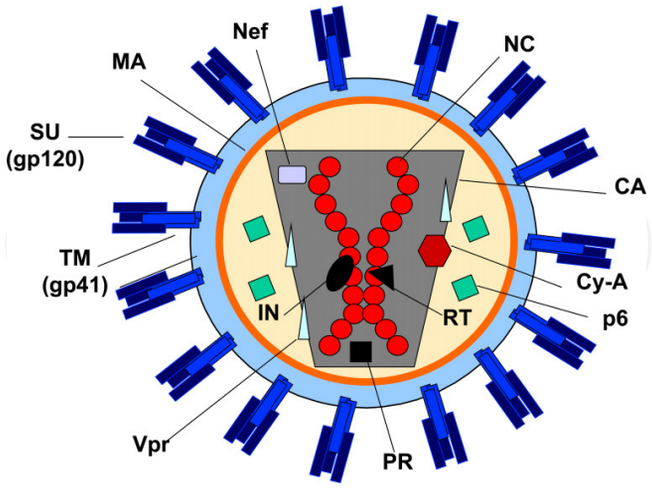

Figure 2. In this scheme, the lentivirus virion is represented as a sphere containing a genome made of two RNA molecules associated to the nucleocapsid (NC) protein. The nucleocapsid is enclosed by a core made of capsid (CA) protein, which is surrounded by a shell of matrix (MA) protein that associates to the virion envelope. The two subunits of the HIV-1 envelope are also indicated (SU and TM). In addition, other enzymatic (IN, RT, PR), accessory (Vpr, p6) and cellular (Cyclophilin A, Cy-A) proteins are shown, which are incorporated into lentivirus particles.

Lentivectors are classified as complex retroviruses according to their genome organisation, as it contains accessory and regulatory genes absent in other retroviruses. Nevertheless, the retrovirus genome shares a common 5′ to the 3′ gene organisation, with Gag, Pol and Env genes [1, 3]. Gag encodes MA, CA and NC as a polyprotein. Pol encodes enzymatic proteins associated to reverse transcription, that is, the reverse transcriptase (RT), integrase (IN) and protease (PR). RT synthesizes a single cDNA copy from the retrovirus genomic RNA [4]. IN mediates cDNA integration in the host cell chromosome, while PR cleaves Gag and Gag-Pol polyproteins during virion maturation.

The integrated cDNA genome is flanked by two long terminal repeats (LTRs) subdivided in U3, R and U5 regions. U3 is the HIV-1 promoter. The R region marks the starting point of transcription, and U5 region is critical for reverse transcription. The other key elements are the packaging signal (Ψ) and the polypurine tract (PPT). The packaging signal, as in many other virus species, allows RNA genome encapsidation during virion assembly in the cytoplasm. The PPT element is a key element for reverse transcription [5].

Lentivectors are usually obtained following a three-plasmid co-transfection in 293T cells (Figure 3) [6, 7]. The first one, the packaging plasmid, provides the structural and RT proteins (Gag-Pol). The second one, the envelope plasmid, encodes a glycoprotein to pseudotype the lentivector particles. This process consists on the incorporation of an heterologous Env in the viral lipid envelope. This will allow the lentivector to exhibit the specific cell tropism given by the Env used in pseudotyping. One of the most used Env is the Vesicular Stomatitis Virus (VSV) Glycoprotein (G). The VSV-G confers stability to the lentivector particles and a very broad tropism for human and non-human cells [8]. Lastly, the third one, the transfer plasmid, contains the cis-acting sequences for replication/transcription and packaging (Figure 3) [9]. By including promoters within the transfer plasmid, any gene of interest can be expressed either constitutively or inducibly, in a cell type-specific of unspecific manner (Figure 3) [1]. Therefore, lentiviral vectors can also incorporate genes with immunoregulatory properties in cells from the immune system.

Two main cell types of the immune system have been preferential targets for genetic immunotherapy: antigen presenting cells (APCs) and effector T lymphocytes. These two cell types are key controllers of immune responses. By expressing transgenes of interest in APCs, such as DCs, they can be processed and presented to antigen-specific T cells in the immunological synapse. This antigen presentation is the first step in either starting of suppressing immune responses. Therefore, if genes with modulating properties of APC functions can be co-expressed with antigens, the strength and type of immune response can be controlled. In fact, genetic modification of cells from the immune system can circumvent the limitations of current immunotherapeutic protocols. Using targeted lentiviral vectors, specificity and effectiveness can be achieved by targeting key cells that modulate and polarize immune responses.

Although more challenging than DCs, T lymphocytes can also be genetically modified using lentiviral vectors. Vectors expressing T cell receptors (TCRs) specific for antigens of interest can modify the specificity of T cell populations, or expand their antigen profile. Therefore, these genetically modified T cells can be adoptively transferred in the human patients. This

strategy is particularly important to generate T cells with high affinity TCRs towards tumour-associated antigens.

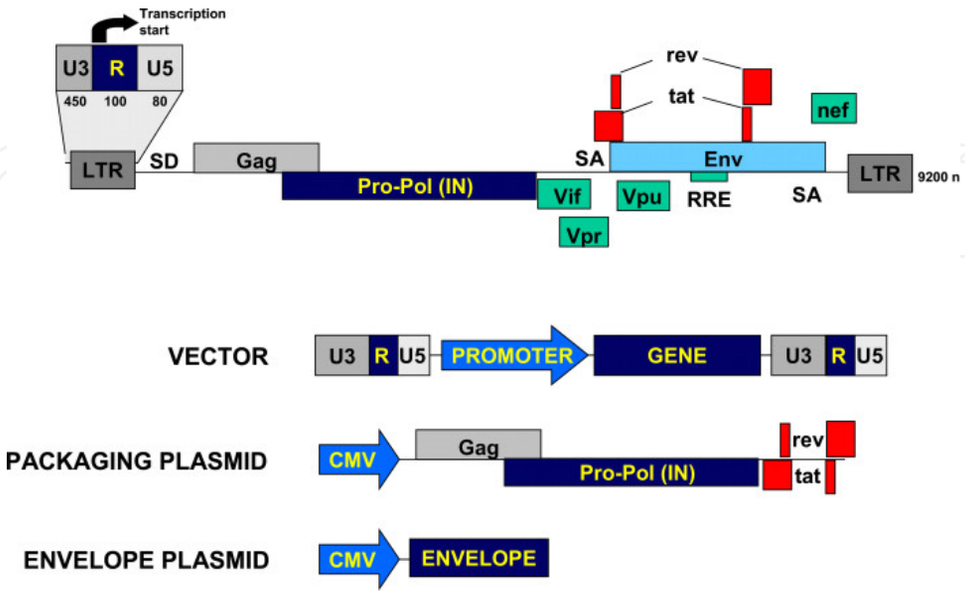

Figure 3. The HIV-1 genome is shown at the top of the figure. All structural, accessory and enzymatic genes are indicated throughout the genome. The two LTRs are shown as present in an integrated provirus. The three functional regions of the HIV LTR are shown on top of the 5- LTR. Numbers indicate nucleotide positions. The HIV genome is splitted in three different plasmids to engineer a gene vector. The transfer (vector) plasmid is indicated, with only the HIV-1 LTRs containing an internal promoter driving the expression of a gene of interest. In the packaging plasmid, only the Gag-Pro-Pol and Rev, Tat genes have been retained. This increases biosafety. Transcription of these genes takes place under the control of the cytomegalovirus promoter (CMV), as indicated in the figure. Lastly, a third plasmid encoding an envelope glycoprotein is shown on the bottom of the figure. This envelope pseudotypes the lentivector particle.

2. Genetic modification of DCs with lentivectors

For the elimination of cancer cells and chronic infections such as HIV, hepatitits B and malaria, a strong, effective T cell response is required. To initiate these strong T cell responses, the interaction between antigen-specific T cells and antigen-presenting APCs has to be strengthened[10]. Amongst APCs, DCs are most frequently the targets of immunotherapy protocols since they are probably the most immunogenic [10, 11].

To activate T cells during antigen presentation, these T cells have to receive at least three different signals from APCs (Figure 4) [10, 12-16]. The first signal, or signal 1, is the direct recognition of the peptide-major histocompatibility molecule complex (p-MHC) by the TCR. However, this interaction is not sufficient to confer T cells with effector activities. For this, a second co-stimulatory signal (signal 2) has to be co-delivered together with p-MHC recognition. This signal 2 is the consequence of the integration of activatory and inhibitory interactions between ligands/receptors on the surface of DCs and T cells (Figure 4)[16, 17]. For example, CD80 binding to CD28 is strongly activatory, while CD80 binding with CTLA-4, or

PD-L1 with PD-1, are strongly inhibitory[16, 17]. Apart from these two signals, T cells require a third signal which drives their differentiation into distinct subtypes that will regulate different types of immune responses [13, 18]. This third signal is usually provided by different combination of cytokines present within the immunological synapse (Figure 4). For example, the presence of high levels of IL12 will polarise T cell differentation towards a Th1 type (cellular cytotoxic immunity). On the other hand, high levels of IL10 will drive polarisation towards Th2 (antibody responses).

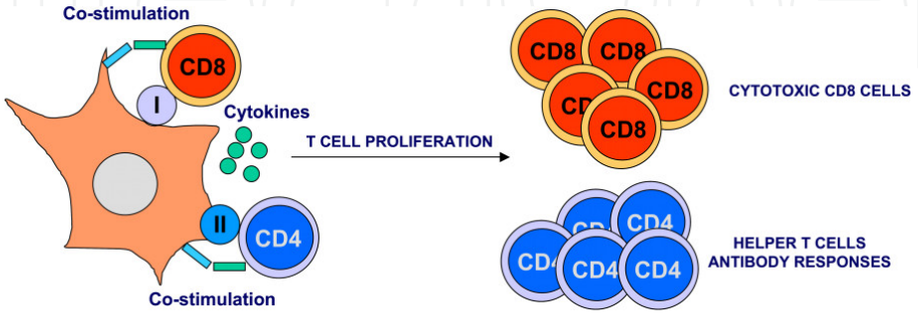

Figure 4. In this scheme, DCs (left) present antigens to specific CD8 and CD4 T cells (as indicated) in the context of MHC class I (I) or class II (II) molecules. These T cells receive further stimuli by co-stimulation through ligand-receptor interactions between the DC and T cells (as indicated in the figure). Simultaneously, activated DCs secrete cytokines and chemokines (indicated in the figure as spheres) that will drive T cell activation, proliferation and differentation into either cytotoxic CD8 T cells or T helper cells, as shown on the right.

An ideal immunotherapeutic approach would be to use lentiviral vectors to deliver the antigen of interest together with the three signals required for the desired T cell polarisation. Lentivectors have been extensively used for this purpose, because they are particularly effective in transducing DCs without affecting their functionality, unlike other vectors such as those based on adenoviruses [8, 19-26]. In fact, the stable integration of the lentivector genome allows long-term, sustained transgene expression[1, 9]. In addition, the expressed transgene is processed and its antigen peptides loaded in MHC I and MHC II molecules [27]. This is of the outmost importance for immunotherapy, since expressed proteins can be processed and loaded onto MHC-II molecules through several pathways. While secreted proteins can enter the endocytic pathway and membrane proteins can be recycled towards the endosomal pathway, cytoplasmic proteins can still enter the MHC II pathway by autophagy[28]. Nevertheless, to improve MHC II loading of peptides from cytoplasmic proteins, endocytic localisation sequences can be fused to the transgene, with the lysosomal-associated membrane protein 1 or with the amino-terminal portion of the MHC II invariant chain [29-32].

The use of lentivectors to express whole trangenes rather than antigen petides circumvents the necessity of designing specific peptide/protein vaccines for loading into specific MHC genotypes [27]. Thus, lentivectors expressing model antigens have been extensively used as a proof of principle. Amongst others, the antigens chicken ovalbumin (OVA), tumour-tumour associated antigens such as MELAN-A, tyrosinase related protein (Trp), NY-ESO or antigens from infectious agents have been expressed in DCs. These modified DCs induced strong activation and proliferation of antigen-specific T cells. [17, 33-38].

3. Lentivector immunogenicity

Lentivectors have been extensively used in vaccination protocols, due to their capacity of raising strong transgene-specific immune responses [9, 17, 30, 38-40]. Interestingly, some reports suggest that lentivectors are incapable of inducing DC maturation *in vitro*, suggesting that some components of the lentivector preparations provide signals 2 and 3 through a mechanisms not well understood [40, 41].

DC maturation is a complex, step-wise process in which they up-regulate the surface expression of co-stimulatory molecules such as CD80, CD83, CD86, CD40, adhesion molecules such as ICAM-1 and also the expression of MHC molecules. In general terms, DC maturation can be triggered by recognition of pathogen-derived molecules by specific receptors on the DC surface, such as the family of toll-like receptors (TLRs) [42, 43]. DCs can also mature through the exposure of pro-inflammatory cytokines by a process called cytokine priming [13-15]. Matured DCs can effectively provide strong signals 1 and 2, leading to efficient T cell activation and proliferation. Thus, their administration *in vivo* induces DC maturation and production of type I interferon that can provide signal 3 [9, 39, 44, 45].

The capacity of lentivectors to induce DC maturation after vaccination is probably caused by either specific components of the lentivector particle or by contaminants present in the lentivector preparation. As a matter of fact, lentivector particles resemble viruses and therefore, some components have the potential to stimulate immune responses such as the RNA genome or the cDNA [46]. These are ligands for TLR7 and TLR9, respectively [41, 45]. In addition to specific components of the lentivector particle, contaminants can also alter their immunostimulatory properties. In fact, most lentivector preparations pseudotyped with VSV G contain VSV-G tubulo-vesicular structures enclosing plasmid DNA that stimulate TLR9 *in vitro*, leading to type I IFN production by pDCs [47]. In addition, foetal calf serum (FCS) contributes to immunogenicity by providing T cell epitopes with adjuvant capacities [48].

4. Control of DC maturation by expression of molecular activators with lentivectors

Lentivector preparations can induce DC maturation in vivo. However, in some circumstances this is not enough for effective therapeutic activities. This is the case for cancer immunotherapy, in which breaking tolerance towards TAAs is still a medical challenge. One possible solution is to co-express TAAs with molecular activators of DCs using lentivectors, particularly using activators of signalling cascades belonging to the TLR pathways.

This has been firstly achieved by over-expressing adaptor molecules, which associate with TLR cytoplasmic tails. These adaptor molecules recruit activatory protein kinases leading to DC maturation. Thus, lentivectors have been used to express MYD88 or TRIF1 in mouse myeloid DCs, which also increases secretion of pro-inflammatory cytokines IL-6, IL-12 and IFN-α, which enhanced T cell cytotoxicity [49]. The NF-κB pathway has also been an attractive target because it controls transcription of the majority of pro-inflammatory genes (Fig-

ure 5). Lentivectors have thus been used to express the Kaposi's sarcoma-associated herpes virus FLICE-like inhibitory protein (vFLIP), a constitutive activator of NF-κB by direct association and activation of NF-κB essential modulator (NEMO) [50-53]. In fact, vFLIP expression has resulted to be a strong adjuvant when expressed in DCs, leading to strong DC maturation and effective CD4 and CD8 T cell responses. Lentivector expression of vFLIP significantly improves anti-tumour activities in a lymphoma mouse model and anti-parasitic efficacy in an OVA-expressing leishmania model [38, 54]. Lentivectors have also been used to inhibit negative regulators of NF-κB activation, such as the ubiqutin ligase A20. Lentivectors have successfully delivered to DCs short hairpin RNAs (shRNAs) targeting A20. The abrogation of A20 expression caused DC maturation, effective CD8 cell responses and inihibition of regulatory T cells (Tregs) [55, 56].

Other molecular activators of mitogen activated protein kinases (MAPKs), activated after TLR engagement, have also been co-expressed in DCs with antigens of interest (Figure 5). MAPKs are mainly divided in three groups, ERK, p38 and JNK. While ERK is associated to survival and immune suppression, p38 and JNK are thought to stimulate DC maturation and inflammation (Figure 5). Constitutive p38 activation was achieved by expressing the MKK6 EE mutant using lentivectors, and it resulted in CD80, CD40 and ICAM-I up-regulation without significant secretion of pro-inflammatory cytokines [17, 40, 41]. A similar result was achieved by JNK1 activation, following expression of the MKK7-JNK1 fusion gene in DCs. Interestingly, although a full DC maturation phenotype was not achieved *ex vivo*, co-expression of these MAPK activators with an OVA-containing transgene or MELAN-A induced significant antigen-specific CD4 and CD8 T cell responses. Moreover, these lentivectors improved survival in a murine tumour model for lymphoma, both with integrating and non-integrating lentivectors [38].

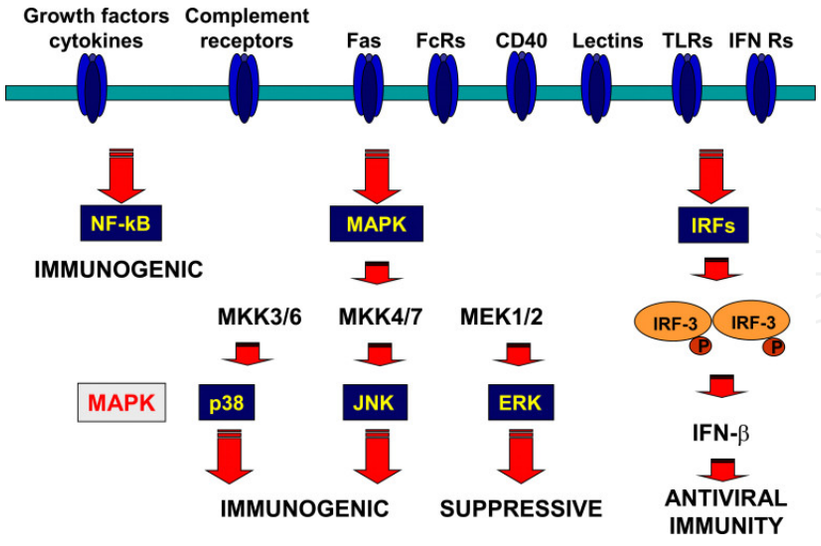

Figure 5. Intracellular signalling pathways regulating DC functions.

Other molecules have been applied for DC maturation. For example, CD40 ligand expression achieved human DC activation and up-regulated the expression of CD83, CD80, MHC-I

and induced IL-12 secretion [57]. This strategy increased CD4 and CD8 responses towards influenza epitopes and the TAA gp100. Co-expression of DC-promoting cytokines such as GM-CSF- and IL-4 using lentivectors resulted in long-lasting immunity against melanoma when co-expressed with TAAs Trp-2 and Mart-1 [58].

In this scheme, the main signalling pathways triggered after the engagement of a wide range of receptors on the DC surface (see the indicated receptors embedded in the membrane) with their ligands. Engagement of these receptors starts a complicated cascade of signalling pathways that will converge in a few, well-characterised ones, the NK-κB, MAPKs and interferon regulatory factors (IRFs) (as indicated below the membrane). Some of these pathways, such as NK-κB, MAPKs p38 and JNK1 are pro-inflammatory and lead to DC maturation. Others, such as ERK, are clearly immunosuppressive.

5. Control of DC maturation by inhibiting negative co-stimulation using lentivectors

DC maturation can also up-regulate molecules that provide negative stimulation to T cells, such as programmed cell death receptor ligand 1 (PD-L1) and PD-L2, the ligands for the PD-1 receptor on T cells. Negative co-stimulation is part of a regulatory mechanism that controls the activation state of T cells following antigen presentation [17, 59, 60]. Thus, interference with negative co-stimulation could in principle reinforce T cell activation and enhance cytotoxic activities. Therefore, lentivectors have been used to deliver shRNAs in DC against PD-L1. PD-L1 silencing in antigen-presenting DCs hyperactivated T cells by preventing the up-regulation of Casitas B-lymphoma (Cbl)-b E3 ubiquitin ligase. This strategy co- accelerated anti-tumour immune responses, particularly if combined with a p38 activator or dominant negative mutant of MEK1, the upstream kinase of ERK [17, 59].

6. Lentivectors and cancer immunotherapy

Lentivectors are particularly promising in cancer immunotherapy, for which conventional immunization is largely ineffective due to two major barriers. Firstly, TAAs are generally self-proteins to which there is strong immunological tolerance. Secondly, that tumours are strongly immune-suppressive and they use several mechanisms to avoid immune responses [41].

Lentivectors can be used in cancer immunotherapy in two different ways. In the first one, DCs can be generated *ex vivo* from the patient, followed by lentivector transduction and *in vivo* administration. Thus, cellular vaccination with transduced DCs expressing HLA-Cw3 induced activation and proliferation of CD8 T cells in a mouse model [37]. Similarly, lentivector transduction was shown to be superior to peptide pulsing in inducing OVA-specific T cell responses [61], protected mice from OVA-expressing tumour cells and significantly inhibited tumour growth. The second strategy is direct lentivector vaccination, taking advantage of their intrinsic immuno-stimulatory capacities and their reduced cost [24, 26, 35].

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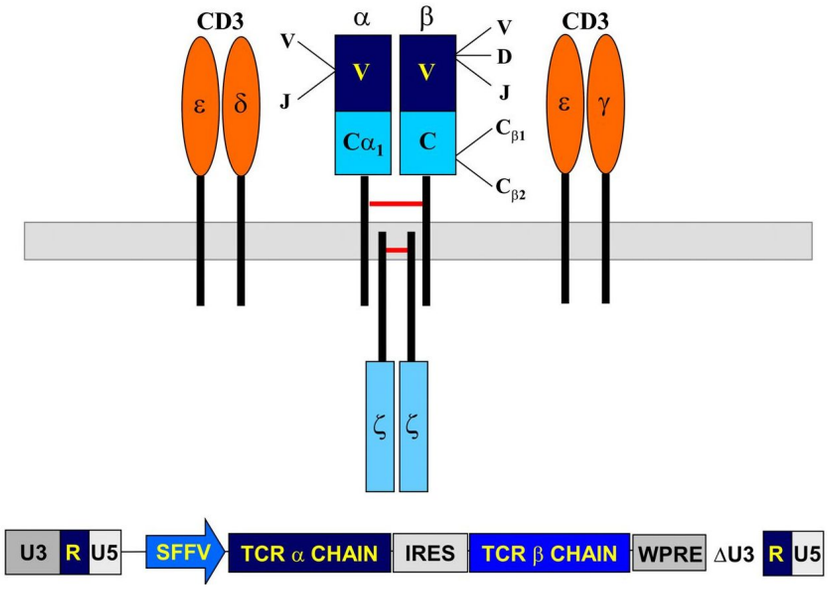

Figure 6. A scheme of the TCR is shown embedded in the cellular membrane. Both α and β chains are shown as indicated, subdivided in variable and constant regions (V, C). The other CD3 chains that associate with the TCR are also included in the figure. On the bottom, a lentivector co-expressing α and β TCR chains is shown, under the control of the spleen focus forming virus promoter (SFFV) and an internal ribosome entry site (IRES) [67]. This particular lentivector is self-inactivating (SIN) and presents a deletion of viral enhancers in the 3' LTR. When this construct is integrated, the 5' LTR disappears and it is replaced with the deleted version.

As mentioned above, a major issue with cancer immunotherapy is that most TAA-specific T cells may have been eliminated during thymic clonal deletion. Thus, even if effective and strong DC maturation is achieved, no effective responses will be achieved due to lack of TAA-specific T cells. To circumvent this, TAA-specific T cells can be generated by lentivector transduction *in vitro*, and adoptively transfered in patients (Figure 6) [62]. Clinical efficacy has been reported for melanoma, synovial cell sarcoma, colorectal, neuroblastoma and lymphoma, but using γ-retrovirus vectors instead of lentivectors [63-66].

T cells are largely refractory to transduction by VSV G-pseudotyped lentivectors, and they require some level of T cell stimulation [67]. Treatment with IL-2 and IL-7 allows lentivector transduction and preserves a functional T cell repertoire [68, 69]. As an example, Wilms tumour antigen (WT1)-specific T cells were generated by lentivector expression of a WT1-specific TCR in the presence of IL-15 and IL-21. These modified T cells were multifunctional and exhibited the expected antigen specificity [67]. This approach of T cell modification is rather promising. In a clinical trial with 15 terminally sick melanoma patients, 2 showed complete regression and long-term survival after transfer of T cells expressing a MART-1-specific TCR using γ-retrovirus vectors [70]. Interestingly, it has been recently demonstrated that entivectors pseudotyped with measles virus H/F glycoproteins effectively transduce quiescent adult T cells in the absence of any exogenous stimulus, whether cytokines or anti-CD3/anti-CD28 stimulation. In fact, transduction with these lentivectors did not affect T cells in any way [17, 71-73].

7. Lentivector gene immunotherapy for the treatment of autoimmune disorders

It is relatively "straightforward" to achieve immune stimulation using lentivectors. However, the induction of immune suppression or tolerance with lentivectors is rather challenging. Nevertheless, the induction and maintenance of immunological tolerance is critical for homeostasis. The organism is permanently and closely in contact with a very wide range of antigens of many origins. A large majority of them are innocuous and do not pose a direct threat. Thus, the immune system must not respond to these antigens, as an immune response is associated with significant collateral tissue damage. The immune system should be activated only if a real threat appears. Therefore, the immune system possesses several tolerogenic mechanisms in place to keep immunological homeostasis. As mentioned before, a key one is clonal deletion of auto-reactive T cells in the thymus [74]. However, there is a significant number of auto-reactive T cells that escape from clonal deletion. Many of them will differentiate towards natural Foxp3 CD4 regulatory T cells [74-77].

In addition to clonal deletion and differentiation of natural Tregs, there are a number of tolerogenic mechanisms in place that regulate immune responses towards peripheral antigens. The organism is in permanent direct contact with many substances and commensal organisms in mucosal areas and in peripheral tissues. In these situations, inducible Tregs differentiate from naïve CD4 T cells after antigen presentation by tolerogenic DCs. These regulatory T cell types are usually classified in Tr1 (CD4 CD25 IL10 or TGF-β) and Th3 (CD4 CD25 Foxp3) cells [78-82]. Therefore, DCs can also be converted in tolerogenic by expression of immunomodulatory genes with lentivectors. This strategy opens up the application of lentivectors for the treatment of autoimmune disorders.

8. Induction of tolerogenic DCs using lentivectors

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9. Induction of tolerogenic DCs using lentivectors

DCs can induce immunological tolerance through a number of mechanisms. It is generally accepted that antigen presentation by immature DCs is poorly immunogenic, and results in Treg differentiation, T cell apoptosis and T cell anergy [83-86]. These immature tolerogenic DCs express low levels of co-stimulatory molecules CD80, CD86, CD83, CD40 and MHC molecules [10, 40, 41, 78, 87]. Resident mucosal DCs are intrinsically tolerogenic independently on their maturation phenotype as a consequence of the presence of retinoic-acid [88]. In addition, these DCs become strongly immunosuppressive due to contact with TLR agonists from commensal microbiota [88-90]. DCs can also become strongly immunosuppressive after treatment with lectin ligands or exposure to immunosuppressive cytokines such as IL-10, IL-4 or TGF-β[78, 87, 89-92]. Tolerogenic DCs usually express high levels of these immunosuppressive cytokines, even if they are phenotypically mature [10, 40, 78, 87, 89-93]. In this situation, they provide strong signals 1 and 2 to T cells, together with a simultaneous strong tolerogenic signal 3. For example, in the presence of bioactive TGF-β, strong antigen presentation leads to differentiation to antigen-specific Foxp3 Tregs, while secretion of IL-10 usually results in Tr1 differentiation [91, 93-95].

Tolerogenic DCs can also up-regulate molecules that provide an inhibitory signal to T cells, such as PD-L1 (or B7-H1), a member of the B7 co-stimulatory molecules [17, 96]. PD-L1 expression in DCs regulates T cell activities during antigen presentation and prevents T cell hyperactivation [17]. In addition, PD-L1-CD80 binding on T cells induces antigen-specific Treg differentiation [97]. Other members of the B7 family are immunosuppressive [98]. Immunosuppressive DCs also up-regulate aminoacid-metabolising enzymes, such as arginase or indoleamine 2,3-dioxygenase (IDO) [99-104]. It is thought that these enzymes deplete T cells of essential aminoacids.

Lentivectors can be used to confer tolerogenic activities to DCs by expression of immunoregulatory genes together with antigens of interest. The first strategy that was tested experimental was the expression of potent immunosuppressive cytokines. This approach was used with γ-retroviral vectors for inflammatory diseases [95, 105, 106], 105, 106]. Lentivectors have been applied in an experimental model of asthma by expressing IL-10, leading to expansion of IL-10-expressing Foxp3 Tregs with potent anti-inflammatory properties [107]. Alternatively, small immunosuppressive peptides can also be delivered with lentivectors, such as the vasointestinal peptide (VIP). Intraperitoneal administration of VIP-encoding lentivec-

tors in mice effectively inhibited the development of experimental collagen-induced arthritis. This was achieved by a markedly reduction of pro-inflammatory cytokine secretion and the expansion of Foxp3 Tregs [108]. The administration of genetically modified VIP-expressing DCs also showed significant therapeutic effects in EAE and in the coecal ligation and puncture (CLP) model [109].

DCs can also be reprogrammed by direct modulation tolerogenic signalling pathways within DCs (Figure 5). Therefore, lentivector expression of a constitutively active MEK1 mutant resulted in sustained MAPK ERK phosphorylation, resulting in immunological tolerance [40, 90, 110-114]. These genetically modified DCs exhibit an immature phenotype with low levels of CD40 and secretion of bioactive TGF-β[40, 78]. Antigen presentation by these ERK-activated DCs differentiated antigen-specific Foxp3 Tregs both *ex vivo* and *in vivo* in a mouse model [78]. Direct lentivector vaccination encoding the ERK activator effectively controlled antigen-induced inflammatory arthritis in a mouse model [78].

Similarly, lentivector expression of a constitutively active IRF3 mutant induced high expression levels of IL-10, and expanded antigen-specific Foxp3 Tregs which inhibited immune responses (Figure 5) [40]. Activation of endogenous negative feedback mechanisms of DC maturation pathways has also been applied to induce immune suppression. In this way, by over-expressing the suppressor of cytokine signalling 3 (SOCS-3) in DCs, pro-inflammatory signalling pathways were severly impaired [115]. These genetically modified DCs significantly decreased secretion of pro-inflammatory cytokines IFN-γ, IL-12 and IL-23, and showed an enhanced IL-10 production, which effectively inhibited effectively inhibit experimental autoimmune encephalomyelitis (EAE) in mice [115].

An alternative strategy to generate tolerogenic DCs is the inhibition of pro-inflammatory signalling pathways instead of activating immunosuppressive pathways. As NF-κB is a critical inflammatory signalling pathway, its inhibition in promising for the induction of immunological tolerance [41]. To achieve this, Rel-B was silenced by the delivery of a shRNA targeted to Rel-B [116]. In this way, its inhibition could effectively prevent DC maturation after engagement with TLR ligands, and it was sufficient to treat autoimmune myasthenia gravis in a mouse model [116]. In an analogous manner, lentivectors have also been applied to silence B cell activating factor (BAFF) in the inflamed joint [117, 118], which was very effective for the treatment of experimental collagen-induced arthritis [119] without the need of targeting the arthritogenic antigen. These lentivectors were directly injected in the inflamed joint, where they preferentially transduced resident DCs. BAFF silencing in these DCs inhibited their maturation, and most importantly, inhibited differentiation of pathogenic Th17 [119].

10. Conclussions

Classical immunotherapeutic strategies for the treatment of cancer and infectious diseases rely on either administration of the antigen peptides together with adjuvants, or the inoculation with attenuated strains of pathogenic agents. This approach has been largely successful for the treatment of a wide range of infectious agents. However, for cancer immunotherapy,

most potent and targeted immunotherapeutic approaches are required to break the natural tolerance towards TAAs. The targeted co-delivery of immunomodulatory genes with antigens of interests to DCs has opened the application of gene therapy for immunotherapy. Lentivectors exhibit a remarkable transduction capacity of DCs and also T cells, and thus, they are ideal tools to achieve immunodulation. In this way, the immune system can be strongly and specifically activated for the treatment of cancer and infectious diseases, but it can on the other hand be strongly immunosuppressed. This makes it possible the induction of immunological tolerance and treatment of autoimmune disorders.

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