Virus Research 176 (2013) 1–15

Contents lists available at SciVerse ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Review

Immune modulation by genetic modification of dendritic cells with lentiviral vectors

Therese Liechtenstein[a,b], Noemi Perez-Janices[a,c], Christopher Bricogne[a], Alessio Lanna[a], Inès Dufait[g], Cleo Goyvaerts[g], Roberta Laranga[a], Antonella Padella[a], Frederick Arce[a], Mehdi Baratchian[a], Natalia Ramirez[d], Natalia Lopez[e], Grazyna Kochan[f], Idoia Blanco-Luquin[c], David Guerrero-Setas[c], Karine Breckpot[g], David Escors[a,b,*]

[a] Division of Infection and Immunity, Rayne Institute, University College London, London, UK
[b] Immunomodulation Group, Navarrabiomed, Miguel Servet Foundation, Pamplona, Navarra, Spain
[c] Epigenetics Group, Navarrabiomed, Miguel Servet Foundation, Pamplona, Navarra, Spain
[d] Oncohematology Department, Navarrabiomed, Miguel Servet Foundation, Pamplona, Navarra, Spain
[e] Cardiovascular Department, Navarrabiomed, Miguel Servet Foundation, Pamplona, Navarra, Spain
[f] Structural Genomics Consortium, Oxford University, Oxford, UK
[g] Vrije Universiteit Brussels, Jette, Brussels, Belgium

ARTICLE INFO

Article history:
Received 18 April 2013
Received in revised form 13 May 2013
Accepted 14 May 2013
Available online 28 May 2013

Keywords:
Dendritic cell
PD-L1
PD-1
Costimulation
Cancer
TCR

ABSTRACT

Our work over the past eight years has focused on the use of HIV-1 lentiviral vectors (lentivectors) for the genetic modification of dendritic cells (DCs) to control their functions in immune modulation. DCs are key professional antigen presenting cells which regulate the activity of most effector immune cells, including T, B and NK cells. Their genetic modification provides the means for the development of targeted therapies towards cancer and autoimmune disease. We have been modulating with lentivectors the activity of intracellular signalling pathways and co-stimulation during antigen presentation to T cells, to fine-tune the type and strength of the immune response. In the course of our research, we have found unexpected results such as the surprising immunosuppressive role of anti-viral signalling pathways, and the close link between negative co-stimulation in the immunological synapse and T cell receptor trafficking. Here we review our major findings and put them into context with other published work.

Contents

Abbreviations: DC, dendritic cell; TAA, tumour associated antigen; NC, nucleocapsid; MA, matrix; CA, capsid; RRE, rev response element; cppt, central DNA flap; WPRE, woodchuck post-transcriptional response element; TCR, T cell receptor; pMHC, peptide–MHC complex; Th, T helper; TLR, toll-like receptor; MAPK, mitogen activated protein kinase; JNK, c-jun kinase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; ERK, extracellular signal-regulated kinase; IRF, interferon regulatory factor; OVA, ovalbumin; X-SCID, X-linked severe combined immunodeficiency; MLV, mouse leukaemia virus; Treg, regulatory T cell; MDSC, myeloid-derived suppressor cell.

* Corresponding author at: Navarrabiomed-Fundacion Miguel Servet, C/Irunlarrea 3, 31008 Pamplona, Navarra, Spain.
E-mail addresses: d.escors@ucl.ac.uk, descorsm@navarra.es (D. Escors).

0168-1702/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.virusres.2013.05.007

1. Introduction

The immune system protects our organism from a variety of threats, including pathogens, toxins and cancer. This protection is achieved through the concerted and regulated action of a number of immune and non-immune cell types. Some of these specialise in antigen capture and processing. These cells decide which of these encountered substances pose a real threat. Other cell types are specialised in neutralising pathogens and toxins, while others exert direct cytotoxicity towards infected as well as cancer cells. Above all, there are systemic homeostatic mechanisms that keep a tight control to prevent collateral damage. For example immune responses directed towards commensal bacteria living in our gut and mucosal areas would be highly detrimental. Thus, understanding the mechanisms of immune modulation in physiological and pathological conditions is essential for the development of novel, effective immunotherapies.

One of the most challenging tasks of the immune system is the protection against cancer. This is a difficult task as in most cases tumour-associated antigens (TAAs) are aberrantly-expressed self-antigens, or mutated versions (quasi-antigens) (Boon and van der Bruggen, 1996; Breckpot and Escors, 2009; Campos-Perez et al., 2013; DuPage et al., 2012; Van den Eynde and van der Bruggen, 1997). Even so, there is accumulating evidence demonstrating the important role of the immune system in anti-cancer activities (DuPage et al., 2012). However, in many instances, immunotherapeutic interventions are necessary to boost these natural anti-tumour activities. Two major barriers have to be overcome to achieve efficient immunotherapy (Breckpot and Escors, 2009). The first barrier consists of breaking the natural immunological tolerance to TAAs. The second major barrier is tumour-induced immune suppression (Breckpot et al., 2003). Tumours are capable of inducing a generalised systemic immunosuppression by a variety of mechanisms, which explains the failure of many immunotherapy treatments. In fact, patients usually undergo immunotherapy in advanced stages of cancer, associated with a strong systemic immune suppression.

The opposite situation occurs in autoimmune disorders. Tolerance towards self-antigens is already broken, and the major challenge is restoring the physiological immune tolerance. This is already a hard task, comparable to raising anti-tumour responses. In many instances the autoantigen is unknown, such as in rheumatic disorders (Flores-Borja et al., 2008). In recent years, palliative therapy for these diseases has improved survival and quality of life, particularly by the application of biological agents which neutralise pro-inflammatory cytokines (Bongartz et al., 2006; Nadkarni et al., 2007).

Dendritic cells (DCs) have been our therapeutic target as they are major regulators of immune responses (Breckpot and Escors, 2009; Escors et al., 2008; Goold et al., 2011) and they control T and B cell responses by stimulating or inhibiting them (Tarbell et al., 2006). DCs are a heterogeneous myeloid lineage of professional antigen presenting cells with high phagocytic capacities and antigen processing/presentation capabilities (Breckpot and Escors, 2009). They patrol peripheral tissues sampling the environment, and after encountering pathogens at sites of inflammation, they take up antigen and undergo a complex phenotype/functional change (maturation). The maturing DCs migrate to secondary lymphoid organs where they present antigens to lymphocytes. Depending on the nature of the particular antigen, as well as the context in which it was found, they will trigger different types of responses (Hawiger et al., 2001). The detailed characterisation of the molecular mechanisms of DC function will help develop methods to effectively control immune responses (Steinman and Banchereau, 2007).

2. Lentiviral vectors to genetically modify DCs

Lentiviral vectors (lentivectors) are excellent tools for biomedical research, as they can transduce dividing and quiescent cells (Escors and Breckpot, 2010; Escors et al., 2012; Goyvaerts et al., 2013). Importantly, lentivectors can accommodate in their lipid "viral" envelopes a wide range of glycoproteins, so they are relatively easy to pseudotype and in this way, control their *in vivo* tropism (Escors and Breckpot, 2010; Goyvaerts et al., 2013). Furthermore, they can stably integrate in the cell genome, leading to prolonged long-term transgene expression. In some cases, integration-deficient lentivectors can also be engineered, when integration is not required, such as in immunisation protocols (Karwacz et al., 2009). This greatly reduces the chances of genotoxicity (Escors and Breckpot, 2010). Finally, lentivectors have been successfully used in clinical trials for the correction of adrenoleukodystrophy, β-thalassaemia, and leukaemia (Cartier et al., 2009; Cavazzana-Calvo et al., 2010; Kalos et al., 2011; Porter et al., 2011). The first clinical trial with therapeutic lentivectors was for the treatment of HIV-1, and it has shown no adverse secondary effects so far (Levine et al., 2006; McGarrity et al., 2013; Waehler et al., 2007). Nevertheless, lentivectors integrate their genome close to transcriptionally active sites, and may cause insertional mutagenesis and gene expression through aberrant splicing (Cesana et al., 2012; Ginn et al., 2010; Knight et al., 2010). Moreover, insertional mutagenesis and genetic instability have been serious genotoxic complications of gene therapy using γ-retrovirus vectors (Hacein-Bey-Abina et al., 2003; Howe et al., 2008; Ott et al., 2006; Stein et al., 2010; Thrasher et al., 2006). Thus, their application in human therapy must be performed with caution.

Lentivectors are usually engineered from the HIV-1 genome. The HIV-1 genome is made of a diploid single-stranded RNA genome that is reverse-transcribed to a single DNA molecule and stably integrates into the cell genome. This integrated version is called a provirus, and consists of a set of genes flanked by two long-terminal repeats which are divided in three functional regions (Fig. 1A); The U3 region is the HIV promoter, followed by the R and U5 regions, required for reverse transcription and efficient gene expression. A key functional element is the packaging signal (Ψ), which is necessary for the specific packaging of the genomic RNA transcript during the assembly of the viral particle. Then, going from the 5′ to the 3′ end, we find the Gag-Pol gene, encoding a polyprotein made up of the nucleocapsid (NC), matrix (MA), capsid (CA)-encoding domains and following a translational frameshift, the protease, reverse transcriptase and integrase enzymes (Jacks et al., 1988; Katz and Skalka, 1994). All these genes are absolutely required for lentivector replication and assembly. The Env gene encodes the HIV envelope glycoprotein, made of two regions, the transmembrane and globular domains. The HIV genome contains other regulatory genes involved in virulence, RNA transcription, processing and transport. From these, the most important ones for engineering lentivectors are rev and tat, required for regulation of splicing and gene transcription (Feng and Holland, 1988; Katz and Skalka, 1994; Rimsky et al., 1988). The presence of these regulatory genes differentiates complex retroviruses (lentiviruses such as HIV-1, HIV-2, spumaviruses) from their simple counterparts such as γ-retroviruses.

The engineering of lentiviral vectors is straightforward. There are several published reviews describing lentivectors and their different generations (Breckpot et al., 2003; Escors and Breckpot, 2010; Zufferey, 2002). Briefly, to construct a lentivector, the majority of the HIV genes are removed leaving the LTRs and packaging signal (Fig. 1B). Other regulatory elements are included to enhance their production, such as the rev response element (RRE) (Daugherty et al., 2010), central DNA flap (cppt) (Sirven