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Review

Problems in targeting dendritic cells with live viral vaccines

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ABSTRACT

Dendritic cells (DCs) are the most effective antigen-presenting cells. Therefore it has been an intriguing concept to target DCs with viral vaccines in order to enhance the immune response against infectious diseases as well as tumor-associated antigens. Unfortunately, live viruses and viral vaccines interfere with cellular functions in many ways. These include pathways leading to apoptosis, impairment of DC maturation by reduced expression of co-stimulatory molecules, and production of cytokines, chemokines etc. This review sums up some of the major findings in the influence of DCs on vaccination and immune stimulation and discusses the obstacles that still remain in the use of different viral families.

KEYWORDS: dendritic cells, DC, virus, vaccine, apoptosis

1. Introduction

Dendritic cells (DCs) have been shown to be the most potent antigen-presenting cells in humans and other mammals. Regardless of minor differences between human and mouse systems, which will be indicated in the manuscript as appropriate, they are uniquely capable of activating the host immune system to elicit a specific cellular and humoral immune response.

DCs were first described by Steinman *et al.* in the early 1970s as phagocytic cells with dendrite-like protrusions. DCs and other phagocytic cells like monocytes, and macrophages have a common precursor, the macrophage-DC progenitor, which differentiates into the common DC progenitor, generating precursor DCs (pre-DCs). These pre-DCs can migrate to lymph nodes, proliferate and differentiate into various DCs. Cells derived from pre-DCs were first classified as “conventional” DCs as they display a classic DC form and function under steady state conditions. Human inflammatory DCs were characterized by their ability to present antigens and to migrate to regional lymph nodes (for a review see [1]).

Additionally, DCs also function as regulatory cells to maintain tolerance to “self” [2]. Aberrant DC expression of surface receptors has been suggested to lead to Th2 polarization, and antigen presentation in the absence of B7/CD28 interaction may result in T-cell unresponsiveness or tolerance [3].

There is no generally accepted nomenclature or staging for the different DC phenotypes and species, presumably due to difficulties in isolating uniquely defined populations. “Conventional” DCs comprise migratory DCs and lymphoid DCs. Migratory DCs are primarily tissue-resident, and once they find an antigen migrate to lymph nodes, where they stimulate T-Lymphocytes. Lymphoid DCs by definition are restricted to lymphoid tissues and are mostly classified according to the expression of CD4 or CD8 surface antigens. Monocyte-derived DCs (CD11c+) and plasmocytoid DCs (CD123+) have been referred to as “non-conventional” DCs. To obtain an efficient anti-pathogen immunity, numerous interactions between DC and T-cell are necessary showing a complex choreography of cellular interactions [4].

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Another crude division of the DCs into two major groups has also been suggested, i.e. plasmacytoid DCs (pDCs) and classical DCs (cDCs), where the cDCs refer to all DCs other than pDCs [5]. The precursors of the classical group derive from common DC progenitors in the bone marrow which migrate *via* the blood to their homing positions in the lymphoid and non-lymphoid tissues. Additionally, blood monocytes can also serve as precursors of inflammatory DCs as it has been shown that in infected tissues they can differentiate into cDCs. Specific DC subsets can be generated from monocytes during inflammation, but they have also been described in steady-state conditions. It is therefore evident that monocytes contribute to intestinal, splenic and muscular cDCs also in uninflammed tissues [6]. pDCs are found in lymphoid and non-lymphoid tissues, and express 2 Toll-like receptors (TLR7 and TLR9) that mediate the expression of interferon regulatory factor 7, a transcriptional activator modulating the production of type I interferons.

In contrast to cDCs, which in most cases seem to be relatively short-lived in most lymphoid organs, pDC appear to be rather long-lived cells. pDCs are also involved both in induction of tolerance and in modulation of autoimmune responses [2]. Antigen-specific T cells have been shown to be capable of destroying DCs, which functions as a kind of negative feedback mechanism for regulation of immune responses. Over-activation of lymphocytes and the onset of autoimmunity have been shown as a consequence of a surplus of DCs resulting from defects in programmed cell death [7].

DC-like macrophages or neutrophils play a role in the innate immune response as well. This system functions mainly *via* host pattern recognition receptors (PRR), which are common to all mononuclear phagocytes. Consisting of various families like Toll-like receptors (TLR), RIG-I-like receptors (RLs) or NOD-like receptors (NLR), PRR react with relatively unspecific pathogen-associated molecular patterns (PAMP), leading to the induction of cytokines, chemokines and type I interferons thereby helping to control spread of the pathogen [8].

2. DC activation and subsequent maturation

DCs have different activities and functions which mainly depend on their stage of maturation. Immature DCs have a very potent phagocytic capacity and can capture and further process antigens, which may then be used to be presented to T-cell/MHC complexes. In contrast to mature DCs, the immature DCs express low levels of MHC-I, MHC-II and co-stimulatory molecules (CD80, CD83, and CD86) on their cell surface. Therefore immature DCs have a relatively low capacity to present antigens to other immune cells. Their role is to patrol in the periphery and to migrate to infection sites, which is facilitated by inflammatory chemokines. This fact is explained by the strong surface expression of chemokine receptors (CCR1, 2, 5, 6, and CXCR1, 2) on immature DCs. Representing two different states of activation, the immature DCs are busy surveying their environment, taking up and processing antigens but have limited capacity to stimulate T-cells, whereas the mature DCs gain the capacity of antigen-presentation, expression of co-stimulatory molecules and efficient T-cell activation [9].

Maturation stimuli lead to a decline in the rate of antigen uptake by DCs. They can be provided by TLR ligands such as TLR4-binding lipopolysaccharide, inflammatory cytokines, or CD40L-mediated help. Thereafter antigen processing is facilitated in mature DCs by the induction of major changes in the endosomal compartments leading to proteosomal alterations and enzymatic activation. In the later stages of DC maturation, genes which are related to antigen presentation are up-regulated. The half-life of MHC-I complexes on the cell surface increases and receptors necessary for the recognition of pro-inflammatory cytokines are down-regulated.

During the maturation process DCs migrate to lymph nodes, where they encounter and activate naive T-cells. To facilitate DC interaction with lymphoid tissues, the expression of several surface receptors is up-regulated, e.g. activation molecules such as MHC-I and MCH-II, and co-stimulatory factors like CD80, CD83 and CD86 are overexpressed in mature DC [9].

DCs also play a crucial role in inflammatory airway diseases by acting against viral, bacterial and fungal pathogens as well as in asthma induced by environmental factors, allergens and microbial products most likely by acting on pattern recognition receptors. There is also a tight interaction between

airway DCs and epithelial cells in the process of initiation of allergic inflammation. Upon exposure to pathogens, allergens or pollutants, inflammatory DCs are recruited to the conducting airways and lung parenchyma. They are characterized as MHCII+ CD11b+ CD11c+ F4/80+ CD64+ and Ly6C+ cells, which appear to be absent at steady state both in peripheral organs and draining lymph nodes, but are readily generated from monocytes. Also, they are the only DC subtype that expresses the high-affinity receptor for IgE (the FceRI) in mice [10].

3. DC vaccines

DCs since their discovery have been considered as potential immunotherapeutic agents that promote the host immune responses against tumor-associated antigens. Several experimental vaccines have been shown to facilitate antigen entry into dendritic cells, which may also provide additional functions of classical adjuvants [11]. However, DC-independent vaccines have also been tested, for example a melanoma cell vaccine which directly stimulates CD8(+) T-cells, without requiring co-stimulatory signals from either CD4(+) T-cells or DCs. In DC-deficient LTa(-/-) mice such vaccines can directly stimulate CD8(+) T-cell responses even in the context of severely reduced DC function [12]. This makes it really difficult to judge the importance of those different mechanisms which most likely occur simultaneously in animal systems as a backup system.

The success of clinical use of such modified antigen-presenting cells has been limited by several factors. Firstly the identification of appropriate tumor-associated antigens exclusively specific for the individual tumors is difficult and may also vary among patients. It is also difficult to maintain DCs in a highly activated state and to achieve an efficient transduction and stable expression of the antigens, without causing major damages, cytotoxicity or unintended functional changes in the transfected DCs. However in 2010, the first DC-based preparation using prostatic acid phosphatase antigen and immune signaling factor granulocyte-macrophage colony stimulating factor (GM-CSF) has been approved for the treatment of prostatic cancer in humans by the US Food and Drug Administration. A summary of recent advances in the preclinical and clinical development of DC-based anticancer therapeutics is given by Bloy *et al.* [13].

4. Reasons for difficulties in virally mediated gene transfer into DCs

The problems associated with virally mediated gene transfer into DCs are fundamental and also apply to the situation found with viral infections. Many of the viruses induce a “host shut off” of the infected cells, in order to obtain a suitable environment for viral replication. Thereby they interfere with a variety of cellular mechanisms, which in many cases are not fully understood. In herpes simplex virus this has been associated with a viral protein contained in the tegument of the virus which can block the induction of DC activation by TLR-independent pathways of viral recognition [14].

As these mechanisms are the evolutionary basis for the success of viruses to replicate in many different host cell types, it is questionable whether they may be easily overcome in DCs, even if the infection in those cells in many cases is abortive rather than productive. Even in incomplete replication cycles it has been experienced that different viruses can prevent the DC activation as well as their maturation. This includes the antigen uptake and processing as well as the antigen-presenting capacity, the production of cytokines, chemokines or interferons, and the expression of surface markers and co-stimulatory molecules. Finally several viruses of different families, as discussed below, have been shown to lead to rapid cytotoxicity in DCs as well as the induction of programmed cell death (apoptosis) utilizing various mechanisms. The extent of the inhibition of immunostimulation by these phenomena is often unclear and it remains to be elaborated whether the mechnisms might at least partially be overcome by the relative resistance of DCs to viral infection. Also the extent of the contribution of other mechanisms like antigen cross-presentation by non-infected bystander cell activation is also not fully elaborated in most studies.

5. Findings with different virus families

5.1. Herpesviruses

DCs can be infected with human herpes simplex virus (HSV) but mature viral particles are only

produced in immature DCs. In mature DCs only immediate/early gene products but not late viral proteins are produced. HSV interferes also with the maturation process and leads to inhibition of the expression of co-stimulatory molecules. This seems to be more or less due to complete degradation of maturation markers like CD86 in lysosomal compartments following HSV infection. Thus HSV fundamentally interferes with the process of T-cell stimulation and DC-mediated T-cell proliferation [15].

As another effective defence strategy, HSV induces apoptosis of attacking DCs. HSV infections of human, macaque, and murine monocytes or bone marrow-derived DC also results in caspase-3 activation and significant DC death [16].

Similar as in cells fully susceptible to HSV replication the cell death in DCs also appears to be a biphasic mechanism with an early phase, in which anti-apoptotic mechanisms are induced, and a late phase with predomination of HSV-mediated pro-apoptotic mechanisms [17].

Although only leading to abortive infection in macrophages and DCs, HSV has been shown recently to lead to the impairment of a broad spectrum of DC functions, especially of integrin and chemokine-mediated chemotaxis and DC migration from sites of infection to the draining lymph nodes [18]. Thus productively infected DCs were not detected in lymph nodes draining the site of infection, which highly suggests that DCs gain access to HSV antigens during infection most likely by uptake from destructed cells rather than as a consequence of being productively infected [19]. Therefore the present concept of HSV skin infection is that viral antigen relay takes place where infected Langerhans cells undergo apoptosis and are taken up by dermal DCs for subsequent antigen presentation [20].

A similar situation has also been observed with human herpesvirus 6 (HHV6) which suppresses DC function without viral replication, and the literature contains sufficient reports on the suppressive effect of HHV6 on T cell proliferation [21]. No significant inhibitory effects by HHV6 infection were observed on co-stimulatory molecules. In the case of immature DCs they have been shown to be up-regulated (CD86) or unaltered (CD40), whereas downregulating effects have been observed on CD44 or DC-SIGN (DC specific ICAM3 grabbing nonintegrin 1). These cell surface receptors are important for homing of DCs and induction of T-cells/maturation, which suggests a reduced ability of HHV6 infected DCs to bind to endothelium and other tissues. Additionally, a clear negative effect of HHV6 has been observed on the cytokine secretion of DCs, especially IL-6, IL-8 and IL-12 whereas the data on IL-10 are more inconclusive (for a review see [21]).

Also human cytomegalovirus (HCMV), another Beta-herpesvirus, has been shown to infect mature and immature DCs *in vitro* leading to suppression of antigen-presenting molecules such as MHC class I and class II, and suppression of co-stimulatory molecules and apoptosis. Among other mechanisms the viral homolog of human IL-10 produced by HCMV has been identified as a strong suppressor of a number of DC functions. Thus the viral IL-10 reduces the antigen-presenting ability of DCs *via* inhibition of anti-apoptotic factors and suppresses secretion of pro-inflammatory cytokines which drives DCs towards a phenotype that could induce T-cell anergy as observed with chronic HCMV infection [22].

5.2. Poxviruses

Infection of DCs with poxviruses also leads to downregulation of co-stimulatory molecules, apoptosis, and cell death. Vaccinia virus (VV) infection leads to an abortive replication in mature and immature DCs and induces apoptotic cell death within a few days. Additionally, maturation of (infected) immature DCs and subsequent T-cell activation are inhibited [23].

This is also observed in DCs infected with parapoxviruses leading to apoptosis presumably *via* CD95(fas)-mediated pathways [24].

Induction of apoptosis in monocyte-derived human DCs detrimental to the subsequent induction of T-cell responses has also been observed with VV strain-modified vaccinia virus Ankara (MVA), which due to adaptation to chicken cells and extended genome deletions is replication deficient in most mammalian cells [25]. Also in the bovine system, MVA-induced apoptosis in DC draining from the skin has been shown to occur soon after

virus binding *via* the caspase 8 pathway and does not seem to be associated with viral gene expression. Moreover, addition of fresh afferent lymph-migrating DCs to MVA-infected cultures did not improve the T-cell responses as these non-infected DCs showed downregulation of MHC class II and co-stimulatory molecules CD40, CD80 and CD86 [26].

Unfortunately these downregulatory and apoptotic effects of VV cannot be overcome sufficiently by adding a broad spectrum of immunostimulatory or anti-apoptotic molecules into the vaccine constructs. Even though it has been previously shown that certain proteinkinase C (PKC) isoforms are clearly involved in DC activation and differentiation [27], using different proteinkinase C constructs in VV constructs did not lead to increased CD86 or HLA-DR expression and also no influence on the maturation of DCs, as measured by DC-SIGN and CD83, was observed [28].

5.3. Retroviruses

Productive infection of human immunodeficiency virus (HIV) has been observed in select populations of DCs in culture, particularly immature DCs derived from blood monocytes and skin (Langerhans cells). However, there exist only a few instances in which HIV- or simian immunodeficiency virus (SIV)-infected DCs have been identified *in vivo* in tissue sections. One of the possible reasons suggested was that they seem to be rather cytopathic in DCs, which has been confirmed by artificial mutants showing less cytopathic effects [29].

The productive HIV infection in DCs seems to be rather restricted, but nevertheless DC play an important role in mediating “trans-infection”, meaning that the DC capture HIV-1 and transfer the captured particles *via* veil-like cytoplasmic protrusions to bystander CD4 T-cells [30]. This mechanism renders tissue DCs an important means of entry, systemic virus dissemination and further progression of HIV infection [31].

HIV directly and indirectly modulates DC function by interfering with the formation of antiviral immunity, but differential dysregulation of myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) has been observed at various stages of the HIV infection. Thus HIV-altered response to Toll-like receptor (TLR) ligands and evasion of innate immune sensing by mDCs leads to suboptimal DC maturation. HIV causes semi-mature, pro-apoptotic mDCs to accumulate in the lymph nodes and DCs taken from lymph nodes or splenic DCs of HIV-infected individuals have shown decreased expression of co-stimulatory markers (CD80, CD83, and CD86), suggesting a link between the lack of DC maturation and the inability of mDCs to stimulate T cells [32]. Altered type I interferon secretion and a weak antigen presentation capacity was also observed in pDCs [33].

A similar situation was observed with SIV where pDCs and mDCs had marked loss of IFN-a and IL-12 production, respectively, and also macrophages lost production of both cytokines [34]. The resulting loss of T-cell stimulating function of macrophage and mDCs in lymph nodes of SIV-infected rhesus macaques has been suggested to be a major mechanism in the development of the generalized immunodeficiency ‘syndrome’.

Integrase-defective lentiviral vectors (IDLV) have been developed by several groups, which can potentially lower the genotoxicity risks using retroviral constructs for gene transfer. Induction of DCs by non-integrating lentivirus, e.g. IDLV co-expressing GM-CSF, IFN-a and the cytomegalovirus pp65 tegument antigen, have been suggested as anti-CMV therapy in stem cell transplant patients [35].

Also, a VPX-containing retroviral DC vaccine has shown promising results, using intracellular expression of CD40-ligand (CD40L) by a viral construct causing transformed(?) DCs to mature and produce a TH1 response [36]. However, the exact role of those experimental vaccines has not been fully elucidated and it seems still unclear whether upregulation of CD80 and CD86 in the untransformed cells may also be predominantly due to the effects of crosspriming.

5.4. Adenovirus

Systemic administration of adenovirus (AV) and adenovirus vectors induces a robust innate and adaptive antiviral immune response in a variety of animal models. AV provides a high-level transduction efficacy for many cell types, regardless of their mitotic status.

AV has been shown to lead to DC maturation, and infection resulted in higher expression of MHC class II, CD54, CD80, and CD86 than CD40 ligation. A reduction in antigen uptake or chemotaxis was also observed [37]. In a mouse model, DC maturation has been suggested to be due to high levels of PI3-kinase-induced TNF-alpha expression by murine bone marrow-derived DCs. However this activation/maturation seems to be caused rather by sensing of viral antigens, e.g. the highly immunogenic AV fiber knobs, *via* integrin binding by uninfected bystander DCs, than a direct viral effect within infected DCs [38].

Recombinant AV serotype 5 vectors lacking E protein expression can be manufactured to high titer and have been extensively tested in human trials of gene transfer and in vaccine studies. However replication-defective AV for gene transfer into DCs has not been overwhelmingly successful so far. This may be due to the development of immunity against the vector itself rather than the inserted oncogen and the pre-existence of antibodies against AV in most humans.

Also a lack of efficiency of expression of the transgenes in certain cell types can be noted. Transduction of DCs with AV at high multiplicity of infection produced transduction efficiencies with 40-45% of the DCs becoming positive for the transgene (p53), and luckily the AV-p53 vector did not adversely affect DC viability [39]. Relative resistance of DCs to AV-mediated gene transfer may stem from a paucity of the cellular receptors that mediate AV entry. Thus AV targeted to CD40 surface receptor has been developed [40], which demonstrated improvements in gene transfer relative to untargeted vectors. However, features of DC maturation observed in that study appeared not to be a function of the AV particle itself, but rather a consequence of targeting to the CD40 activation marker.

5.5. Myxoviruses: Paramyxoviruses

Paramyxoviruses have been shown to replicate in monocyte-derived human DCs. Parainfluenza 3 virus (PIV-3) not only inhibits interferon signaling, like many other viruses, but also leads to apoptosis in the vast majority of the infected DCs. However, the surviving (uninfected?) DCs seem to undergo robust maturation as measured by upregulation of co-stimulatory molecules [41]. In general the data on co-stimulatory molecules are rather varying among paramyxoviruses. In contrast to findings of partial DC maturation with PIV-3 or reports of no effect using PIV-5, which failed to induce any co-stimulatory molecules, RSV, metapneumovirus, and some strains of measles virus seem to induce both CD80 and CD86 expression in human monocyte-derived DCs. Also the data on cytokine and chemokine production are varying and hampered by the fact that it is not clear in most studies, whether they are produced from infected or uninfected DCs (for a review see [42, 43]).

Also, measles virus (MV) interferes with DC maturation. A decreased expression of MHC and co-stimulatory molecules and lack of expression of chemokine receptor CCR7 were observed. Infection resulted in apoptosis in DC co-cultures with T-cells, which may contribute to a reduced T-cell response and MV-induced general immunosuppression [44].

5.6. Flaviviruses

Resident dendritic cells and Langerhans cells in the dermis are the first cell targets of dengue virus (DENV) infection. Human monocyte-derived DCs (moDCs) generated *in vitro* support DENV infection, with immature moDCs being more susceptible to DENV infection than mature moDCs, monocytes or macrophages. DC-SIGN seems to be one of the lectins mediating this susceptibility. DENV has also been reported to impair DC activation and subsequent priming of adaptive T-cell responses [45].

In humans, DENV suppresses the interferon alpha/beta response which is not observed in murine models despite susceptibility of murine macrophages and monocytes for DENV [46]. The lack of interferon leads to reduced stimulation of DC activation, and DENV infection additionally leads to apoptosis resulting in impaired antigen processing and presentation functionality of DENV-infected DCs.

Other members of the Flaviviridae, e.g. Hepatitis C virus (HCV), Swine fever virus, also have been shown to induce DC apoptosis. This was attributed to the expression of the F protein of HCV which has been shown to lead to apoptosis in DCs,

presumably through Fas-ligand expression. However, endogenous expression of F protein in human DCs has lead to contrasting effects on activation and apoptosis, allowing activated DCs to efficiently internalize apoptotic DCs, thus providing a major tool of clearance of the virus [47].

Bystander apoptosis was observed even in DENV-uninfected DCs, indicating that virus replication may not only induce DC apoptosis directly but may even kill uninfected neighbor cells through the action of cytokines (e.g. IL-10), viral proteins or exosomes secreted by the infected DCs [48]. However, somewhat contradictory observations of an increased survival of non-infected bystander cells suggest that the virus certainly blocks activation in infected DCs, which may decrease the priming of CD4 or CD8 T-cells, whereas non-infected bystander cells still can become activated [45].

6. Possible effects of crosspriming

Cross-presentation of antigen to cytotoxic (CD8+) T cells appears to be an important mechanism for the development of specific cytotoxic T lymphocyte (CTL) responses against tumours and viruses which do not directly infect the antigen-presenting cells. In the mouse, specialised subtypes of DCs have been suggested as being necessary for antigen cross-presentation, and also human homologues of these DCs have been recently found (for a review see [49]). Their functions could be the explanation for the paradox that while vaccinia virus inhibits DC maturation and also causes extensive apoptosis of infected cells, including abortively infected DCs, it has been shown highly immunogenic. Moreover (uninfected?) DCs were found to cross-present antigens from both apoptotic and necrotic infected DC or Langerhans cells (LCs) to CD8(+) T cells. This is probably due to the effective uptake of dead cells by non-infected immature DCs and following exposure to maturation stimuli, especially CD40 ligand. By this means of cross-presentation of vaccinia-derived antigens from infected apoptotic DCs or necrotic cell lysates, the deleterious effects of direct infection of DCs are overcome, which provides one possible explanation for this pathogen's undisputed and proven immunogenicity [50].

The same situation comes true with human herpesviruses and there are several lines of evidence in support of the view that CD8+ DCs gain access to viral antigens from exogenous sources rather than as a consequence of being productively infected. Langerhans cells (LCs) are the first DCs to come into contact with HSV antigens in the skin, but it is CD103(+) dermal DCs that carry viral antigen to lymph nodes for antigen presentation, suggesting extended cross-talk by DCs in skin. HSV-infected LCs rapidly undergo apoptosis and are taken up by dermal DCs for subsequent antigen presentation [20].

However, definitive proof that cross-presentation is required for HSV-specific CTL priming is still missing and the exact contributions of direct action versus cross-presentation to the overall magnitude of anti-viral T cell immunity still remains to be unraveled.

It is well accepted that cross-presentation of antigens is a key feature of DCs. Most recently also a role of invariant natural killer cells in the cross-talk with cross-priming DCs and memory T-cell formation has been suggested [51].

7. The role of apoptosis

Apoptosis of DCs has an important role in immune regulation, because it controls processing and availability of antigen to T-cells. Therefore any significant alteration in the rate of cell death in DCs has a major effect on the antigen-specific immune response, inflammation and immune tolerance. Programmed cell death has been shown to be essential for the maintenance of lymphocyte homeostasis and immune tolerance. Apoptosis plays an important role in regulating spontaneous DC turnover and it appears that both over-accumulation and depletion of DCs can disrupt immune tolerance [7]. There is also increasing evidence that DC-mediated immunoregulation has an important role in the development of autoimmunity [52].

It is therefore conceivable that there are several mechanisms preventing over-stimulation by DCs. For example in the absence of feeding cytokines like GM-CSF or IL-4, DCs undergo spontaneous apoptosis. IL-10 induces cell death by down-regulating anti-apoptotic proteins such as Bcl-2

and TGF-ß. Also type 1 interferons have been shown to lead to apoptosis in DCs [53].

Thus it seems very likely that the decay of non-stimulated immature DCs has a significant role in the regulation of the immune response, which is obviously used by many successful virus groups, and the disruption of DC function may therefore be also a general principle of viral persistence [54].

8. Conclusion

A review of the literature shows that several virus classes have developed similar mechanisms to avoid immune-presentation by DCs. This occurs by interacting with endocytic pathways and antigen presentation mechanisms provided by macrophages and dendritic cells (for a review see [55]).

Also rather simple but highly effective strategies like causing programmed cell death in infected DCs are found in different virus families. It is now widely accepted that the maintenance of DC homeostasis by programmed cell death has major impacts on the antigen-specific immune responses and the balance between immune tolerance and autoimmunity [7]. Major effects of virus infection on DC numbers and function therefore would certainly have detrimental effects on the immune system.

It seems that interference with the DC maturation process as well as the influence on the expression of surface markers, cytokines and chemokines or their receptors are additional common features among viruses, which are all necessary for the immunostimulatory capacity of DCs. Most importantly, this affects MHC molecules necessary for antigen presentation as well as several co-stimulatory molecules needed for subsequent activation of T-cells. However data are confusing sometimes and different results have been obtained with animal and human DCs under similar conditions. This puts a question mark over the validity of animal models for studying human DC function.

Additionally, due to the limitations in the experimental setup in most studies it is also not possible in many cases to clearly discriminate between direct effects from infected DCs and bystander activation of uninfected DCs. Concerning the efficiency of the antiviral immune response, and the level and duration of protection, it has been assumed that live attenuated vaccines are superior to non-replicating vaccines. This comes true in many cases, although there are several exceptions to the rule (reviewed in [56]).

Viral strains modified by gene techniques or non-replicating viral vectors, which have not been the topic of this review, as well as particle-antigen conjugates have been suggested for use in next generation vaccines. The scientific principle behind such vaccines in most cases is to present the antigen in combination with targeting other cellular functions, especially of DC, in order to provide additional stimuli for cell activation and maturation. Mostly consisting of growth factors or cytokines, these maturation signals should enhance the immunogenic capacity of the “old” vaccines to function as intrinsic adjuvants acting on antigen-presenting cells (for a review see [11]).

However, the interfering viral mechanisms seem to be rather fundamental in the sense that they can hardly be overcome by the addition of broad-spectrum co-stimulatory or anti-apoptotic proteins to live viral constructs [28]. Such virus-mediated immune-modulating mechanisms could account for the poor clinical responses in patient trials targeting tumor cells or chronic viral diseases, which so far in most cases have not resulted in a dramatic increase in patient survival rates [49].

In summary, although high transduction efficacies of DCs were obtained with several viruses, the complexity of virus/cell interactions have revealed a variety of mechanisms that interfere with crucial functions of DCs *in vivo*. Their complexity and fundamental role in cell activation, maturation and apoptosis have largely destroyed the initial optimism in the field of DC transduction for vaccination purposes using live viral strains or modified viral constructs with functional replication activity.

CONFLICT OF INTEREST STATEMENT

The author declares that there is no conflict of interest regarding the publication of this paper.

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