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Dendritic cells in immunotherapy of established cancer: Roles of signals 1, 2, 3 and 4

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Abstract

Despite the ability of cancer vaccines to induce tumor-specific T-cells in the blood of patients with cancer, and early, promising data indicating their ability to delay cancer progression, their ability to induce cancer regression remains low. The use of ex vivo-generated dendritic cells (DCs) in such vaccines can help to sidestep the cancer-associated dysfunction of endogenous DCs and to deliver the key instructive signals needed for effective antitumor responses. Effective ways of loading DCs with tumor-related antigens, while retaining the high costimulatory function required for T-cell expansion (ie, effective delivery of 'signal one' and 'signal two'), have been previously identified. More recently, different DC populations have been found to deliver a specialized third signal, able to regulate the acquisition of desirable T-cell effector functions, as well as an additional fourth signal that regulates the homing properties of T-cells. Moreover, ex vivo instruction of DCs can be used to preferentially activate CTLs, T-helper 1 and NK cells, while limiting the undesirable activation of regulatory T-cells. These developments can result in the induction of T-cells with desirable effector functions and tumor-relevant homing properties, even in the absence of proinflammatory signals (typically present in recall infections, but not in advanced cancer), thus helping to bridge the gap between the effectiveness of therapeutic and preventive cancer vaccines.

Keywords

Cancer; chemokine; CTL; cytokine; dendritic cell; IL-12; immunotherapy; NK cell; Th1; vaccine

Introduction

Despite advances in cancer prevention and therapy and the recently noted decrease in cancer-related deaths in the annual report from the American Cancer Society, CDC, NCI and the North American Association of Central Cancer Registries, cancer remains a leading cause of mortality [1,2], with substantial numbers of patients lacking effective treatment and even larger numbers lacking definitive cure. The combined use of surgery, radiotherapy and chemotherapy is often highly successful in eliminating the major tumor mass, but is less effective in eliminating residual cancer cells and in preventing disease recurrence. This particular disadvantage of the currently available treatments has provided the rationale for utilizing the immune system (specialized in eliminating 'rare events' in our bodies, such as invading bacteria or host cells hijacked by viruses) in a therapeutic context to identify and destroy cancer cells.

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Therapeutic cancer vaccines attempt to instruct the patients' own immune system to kill cancer cells. They have the unique advantages of low toxicity and of being able to target multiple target molecules, even newly arising antigens on rapidly-mutating tumor cells. Following the initial demonstration by William Coley that the immune system can be mobilized to fight established cancer, large research efforts have helped to understand the basic principles governing immune recognition and elimination of tumor cells [3].

Recent observations that cancer vaccines, particularly dendritic cell (DC)-based vaccines, are able to induce 'epitope spreading', that is, to extend the antigenic spectrum of responses beyond those observed with the original vaccine [4,5], further support the ability of vaccines to target heterogeneous tumor-cell populations, despite the adaptability and antigenic mimicry associated with such cells.

However, despite the increasingly high immunological effectiveness of new cancer vaccines and indications of their ability to delay cancer progression [6], currently available therapeutic vaccines against cancer are still not as effective as preventive vaccination is against infective agents [6–14]. In particular, while current cancer vaccines exhibited early promise in inducing disease stabilization and prolonging patients' survival [12,15–17], they have remained poorly effective in inducing regression of bulky tumors [11].

Therapeutic vaccines: Old paradigms and new challenges

Compared with preventive vaccines, which aim to induce the expansion of pathogen-specific T-cells and to establish immune memory, therapeutic vaccines need to successfully overcome several challenges that are unique to the setting of established cancer (Figure 1). In contrast to responses to tissue-invading microorganisms mediated by immunological memory, vaccination-induced T-cells in patients with cancer are not exposed to proinflammatory danger signals from infected tissues and innate immune cells, which are known to facilitate the development of T-cell effector functions and their attraction to the sites of pathogen entry [18–20]. This introduces more stringent requirements for therapeutic vaccines, which in addition to driving the expansion of cancer-specific T-cells, must also directly induce the acquisition of T-cell effector functions and tumor-relevant homing potential.

The immune suppression associated with advanced cancer and the dysfunction of endogenous DCs and other APCs [21–23], whose function is to stimulate effector T-cells, as well as the hyperactivation of regulatory T-cells (Tregs) [24–27], which are able to suppress active immunity, are additional factors that need to be taken into consideration in therapeutic vaccine development. Endogenous DCs in cancer-bearing patients are a target of tumor-associated suppressive factors, resulting in their aberrant function and thus, the impaired induction of effector activity in tumor-specific lymphocytes [28,29]. Mediators of tumor-induced DC dysfunction include IL-10, TGFβ, VEGF, IL-6 and prostanoids, such as PGE_2, generally overproduced in cancer [21–34]. DCs developing in the presence of such factors fail to mature, and are thus unable to express sufficient levels of costimulatory molecules required for T-cell activation, or to produce the cytokines required to support the survival and effector functions of tumor-specific T-cells [35–38]. Dysfunction of endogenous DCs has been observed in patients with melanoma, ovarian, breast, renal, prostate, lung and head and neck cancer [36,39–43]. The absence of adequate costimulation and cytokine secretion by DCs leads to naïve, memory and effector T-cell anergy, thus favoring tumor evasion [30–34]. In addition to the dysfunction of endogenous DCs, patients with advanced cancer often show expansion and hyperactivation of Tregs [24–27]. Tregs limit the effectiveness of cancer vaccines [26,44], and they have been found to be preferentially expanded in the presence of at least some of the currently used cancer vaccines [27].

The above observations raise concerns as to whether the traditional vaccine paradigms, developed based on the experience with current protective vaccines, are relevant to, or sufficient for the development of therapeutic vaccines against cancer. Even though, similar to preventive vaccines, therapeutic vaccines need to be effective in inducing the expansion of cancer-specific T-cells, as well as their subsequent development into memory cells, they must also be effective in overcoming tumor-associated immune dysfunction/suppression and may need to 'adopt' the role of proinflammatory cytokines and chemokines (typically present in infected tissues) in inducing tumoricidal effector functions and tumor homing.

Ex vivo-generated dendritic cells in cancer treatment: Dendritic cell-based cancer vaccines

The dysfunction observed in endogenous DCs in patients with cancer suggested the use of *ex vivo*-generated DCs as carriers of cancer vaccines [45]. DCs, originally identified by Ralph Steinman in 1975 [46–49], are APCs uniquely specialized in inducing primary immune responses, supporting the survival and effector functions in previously primed T-cells and mediating overall communication within the immune system [50,51]. As, in contrast to DCs that develop in the context of tumor-related suppressive factors, fully mature DCs acquire at least partial resistance to such mediators [52–54], *ex vivo*-generated DCs have been extensively used as a therapeutic tool.

Selecting the right tools for cancer vaccines: Dendritic cells as carriers of signals 1, 2 and 3

DCs provide T-cells with an antigen-specific 'signal 1' and a costimulatory 'signal 2' [55–57], both of which are required for the activation and expansion of pathogen-specific T-cells. DCs also provide an additional third signal (signal 3), which polarizes the development of immune responses toward T-helper-1 cell (Th1) or Th2 responses (type-1 or type-2 immunity, which is desirable and undesirable in cancer, respectively) [51], thus leading to differential activation of particular effector mechanisms, as well as different capabilities in inducing cancer rejection [51,55–64]. In addition to their role as initiators of antigen-specific Th responses, DCs also activate and support the tumoricidal functions of NK cells [65,66]. Also, effective induction of antitumor CTL responses requires mature DCs expressing high levels of costimulatory molecules and that are able to migrate in response to CCL19 (chemokine [C-C motif] ligand 19) or CCL21, the lymph node-produced CCR7 (chemokine [C-C motif] receptor 7) ligands [67–70]. In addition, high IL-12p70 secretion dramatically enhances the ability of DCs to induce tumor-specific Th1 and CTL activation, and to promote tumor rejection in therapeutic mouse models [71–81].

Unfortunately, obtaining DCs with high immunostimulatory function, high migratory activity and high capacity to produce IL-12p70 proved to be difficult. First-generation DC-based vaccines utilized relatively immature or partially mature DCs, which were immunogenic and able to promote stabilization or regression of cancer in a proportion of patients [82,83], but were suboptimal with regard to their lymph-node homing ability and T-cell-stimulating potential [67,68]. Second-generation DC-based vaccines used cells which were fully mature in terms of costimulatory and homing function (matured in the presence of an IL-1β/TNFα/IL-6/PGE_2-containing cytokine cocktail [84]), but exhibited a reduced ability to produce bioactive IL-12p70, a phenomenon referred to as 'DC exhaustion' [52,85,86]. Thus, although second-generation DC-based vaccines were clearly superior to the immature/partially mature DCs used in the first-generation vaccines with respect to their immunogenic capacity [67,68] and migratory responses to lymph node-associated chemokines [70,87,88], the combination of these two properties with the production of IL-12p70 was difficult to attain.

Based on the above observations, the feasibility of inducing 'non-exhausted' mature DCs was examined as a means of boosting the clinical efficacy of cancer vaccines [53,86,89–95]. More specifically, immature DCs were exposed to Th1- and Th2-associated IFNs and T-cell receptor (TLR) ligands, or, alternatively, to properly-activated NK cells or CD8+ memory T-cells. The resulting 'type-1 polarized' DCs (DC1s) exhibited a dramatically enhanced capacity to induce long-lived tumor-specific T-cells with strongly pronounced antitumor effector functions in both human *in vitro* and mouse *in vivo* models, as well as the ability to enhance tumoricidal functions of resting NK cells. The original observations from the Pawel Kalinski research group [53,86], in combination with data from the laboratories of Brian Czerniecki in Philadelphia, PA, USA [93] and Marieke van Ham in Amsterdam [96], demonstrated that culturing DCs with a combination of IFNγ and LPS (including monophosphoryl lipid A, the clinical-grade form of LPS) or in the presence of the maturation-inducing cytokines TNFα and IL-1β was able to overcome the maturation-associated 'exhaustion' of the cells, yielding stable DC1s able to produce highly elevated levels of IL-12p70 upon interaction with CD40L-expressing (ie, activated) CD4+ Th cells and to induce more potent Th1 and CTL responses (as assessed by increased tumor-cell recognition) [53,93]. DC1s with similar properties can be effectively induced by 'two-signal-activated' autologous NK cells or CD8+ memory T-cells ([91,92,95] and [Kalinski P: unpublished data]). Further addition of IFNα and polyinosinic:polycytidylic acid (poly I:C) to the DC maturation cocktail enhanced the ability of maturing DCs to acquire CCR7 expression [86], and instructed the cells to preferentially interact with naïve, memory and effector T-cells, rather than with Tregs [34] (see below). These results suggested that polarized DCs may be able to avoid the undesirable expansion of Tregs observed with previously used cancer vaccines [27,97–100].

In accordance with the ability of polarized DCs to induce qualitatively improved immune responses, 'α-type-1-polarized' DCs (αDC1s; cultured in IFNα/poly I:C/TNFα/IL-1β/IFNγ) induced higher numbers of long-lived, functional, melanoma-specific CTLs (on average 20-fold higher) following a single round of *in vitro* sensitization [86], when directly compared with standard (s)DCs, matured in the presence of IL-1β/TNFα/IL-6/PGE_2 [84], a maturation protocol frequently used in second-generation DC-based vaccines. Data from melanoma [86], chronic lymphocytic leukemia [101], and several other cancers, uniformly demonstrated the feasibility of generating DC1s from patients with advanced cancer and loading them with peptide antigens [86] or apoptotic tumor cells [101] to induce tumor-specific CTLs.

Based on the promising results from the first clinical trials involving therapeutic vaccines using partially mature first-generation DCs in follicular lymphoma and melanoma [82,83] in the mid-1990s, DCs have since been used to treat patients with several different malignancies. Even though the rates of clinical responses (as measured by RESIST [response evaluation criteria in solid tumors] or WHO criteria) rarely exceed 10 to 15% [11,15–17,45,102], recent data from phase III clinical trials of a first-generation DC vaccine against prostate cancer (Sipuleucel-T/Provenge, Dendreon Corp) demonstrated that it prolonged the overall survival of vaccinated patients [16,103,104], thus raising the question of whether clinical responses (measured by RESIST criteria that were developed to monitor the direct cytotoxic effects of chemotherapeutic agents) can accurately predict the long-term advantage of cancer vaccines [6,13].

Intriguingly, while second-generation DC vaccines, matured in the IL-1β/TNFα/IL-6/PGE_2-containing cytokine cocktail [84], are clearly superior to immature DCs with respect to their immunogenic capacity [67,68] and migratory responses to lymph node-associated chemokines [70,87,88], as previously discussed, a recent phase III clinical trial of autologous, peptide-pulsed DCs in advanced melanoma demonstrated that their

effectiveness (< 10%) was not superior to dacarbazine treatment [105]. The reason for this disappointing result remains unclear; however, the undesirable negative impact of PGE_2 on the production of IL-12 [34,53,54,106], a cytokine which has numerous activities central to the induction and survival of type-1 immune cells [61], and the susbequent activation of Tregs [27,98] are possible culprits in this respect.

The clinical efficacy of third-generation DC-based vaccines using DC1s is currently being evaluated at the University of Pittsburgh Cancer Institute, in clinical trials in cutaneous T-cell lymphoma, glioma, melanoma and colorectal cancer (ClinicalTrials.gov identifiers: NCT00099593, NCT00766753, NCT00390338 and NCT00558051, respectively), with early clinical data expected to be available in 2010, whereas a trial in prostate cancer was expected to begin shortly after the time of publication [G Chatta, personal communication]. While the IFN-induced type-1-polarization of DCs, used to avoid the 'exhaustion' of mature DCs [53,86,93,96], offers a clinically applicable way of enhancing the desirable features of DCs used as cancer vaccines, the effectiveness of this approach needs to be compared (or possibly combined) with other ways of enhancing the desirable properties of DCs. Such potential alternatives and combinations may include the use of IL-15 (rather than IL-4) to promote early stages of DC development [107], the use of B7-DC cross-linking [108], inhibition of p38MAPK [109,110] as alternative ways of enhancing T-cell activation, or inclusion of multiple TLR ligands that can have a synergistic effect in the induction of bioactive IL-12p70 and prime DCs for high IL-12 production during the interaction with T-cells [111–114], thus generating DCs able to produce high levels of proinflammatory cytokines, as well as having other desirable features, as discussed below.

DCs as mediators of signal 4: Induction of tumor-specific T-cells with tumor-relevant homing potential

Differences in the homing properties of different T-cell subsets have been known for some time [115–121], but it was only in the last 6 to 7 years that it became apparent that DCs play an important role in the regulation of T-cell homing characteristics [122–126]. DCs use vitamins A and D to induce the T-cell chemokine receptors, CCR9 [127] and CCR10 [128], thus allowing T-cells to preferentially migrate to the gut or the skin. In addition, DCs from Peyers' patches or DCs treated with vitamin A derivatives are able to induce gut-homing properties in T-cells [123–126]. It was also recently established that migratory APCs imprint the integrin-mediated ability of T-cells to home to the CNS [129]. It may therefore be hypothesized that DCs and different DC-based vaccines can affect the homing abilities of tumor-specific T-cells, exhibiting differential abilities in terms of directing them to different tumors and other tissues.

In support of the notion that the migratory capacity of human, cancer-specific T-cells can be affected by DC-related factors (delivery of 'signal 4'), enhanced expression of functional cutaneous homing receptor (the ligand for the skin endothelial leucocyte adhesion molecule) and enhanced migration of effector CTLs to metastatic melanoma lesions in the skin could be induced by the treatment of patients with systemic IL-12 [130]. It has also been demonstrated that vaccination with monocyte-derived DCs can induce melanoma-specific T-cells that home to both the skin and visceral metastases [131].

The possibility that improved tumor homing may translate into better outcomes with active immunotherapies is supported by the observation that the level of T-cell infiltration is a strong independent prognostic marker of the survival of patients with melanoma [132] and colorectal cancer [133–135]. A dramatic survival advantage associated with CXCR3 (CXC chemokine receptor 3) expression by CTLs has also been observed in patients with advanced melanoma [136]. Therefore, the ability of DC vaccines to induce CXCR3-expressing CTLs

is likely to contribute to their ability to act as effective vaccines against melanoma and potentially other tumors.

DC-produced chemokines: Avoiding regulatory T-cells and directing vaccination-induced effector cells to tumors

In addition to the ability of DC-based vaccines to induce desirable effector functions and the expression of a defined set of homing receptors on tumor-specific T-cells, another aspect that needs a thorough evaluation is the possibility to manipulate tumor-infiltrating DC vaccines to selectively express chemokines that attract (and thus preferentially activate) appropriate types of immune cells, such as Th1, NK cells and CTLs, while avoiding interaction with suppressor/regulatory cells. It was recently demonstrated that the conditions under which DCs mature imprint the ability to secrete different classes of chemokines as mature cells and thus, to selectively attract and interact with functionally distinct T-cell subsets [34]. DCs matured in the presence of PGE_2 preferentially secrete CCL22/MDC (macrophage-derived chemokine) and attract Tregs [34], which may explain the previously reported preferential expansion of undesirable Tregs in patients vaccinated with PGE_2-matured sDCs [27]. In contrast, the inclusion of IFNs in the DC maturation cocktails, particularly when combined with the absence of PGE_2, suppresses CCL22 production and promotes the secretion of effector T-cell-attracting chemokines, such as CCL5 and CXCL10 (chemokine [C-X-C motif] ligand 10), as well as other CXCR3 ligands [34]. This ability to produce specific chemokines seems to be imprinted during maturation, as the chemokine expression remains stable even after removal of the original maturation stimulus [34]. Therefore, it is possible that the use of DCs matured in different environments, such as PGE_2-matured sDCs and αDC1s matured in the presence of IFNs and other NK cell-replacing factors, by mimicking the conditions of acute infection, will preferentially amplify functionally different types of immunity.

As several tumor microenvironments are rich in PGE_2 [30–33] and CCL22 [25], and/or are able to effectively recruit Tregs [25] rather than effector T-cells, it remains to be tested whether functional modulation of intratumoral DCs may reduce CCL22 levels and Treg infiltration. The possibility that tumor-specific chemokine modulation may enhance the overall effectiveness of cancer immunotherapy is indirectly supported by studies demonstrating that DCs in regressing tumors exhibit particularly high levels of CXCL9 expression and effectively attract CXCR3+ T-cells [137,138], and will be directly tested in upcoming clinical trials in colorectal cancer and melanoma.

Conclusion

Data from clinical trials of cancer vaccines, including first-generation DC-based vaccines, have suggested that such therapies may delay tumor progression and prolong the survival of patients with advanced cancer [16,17,103,104]. However, their activity in inducing tumor regression is still limited.

In addition to the more traditional role of DCs as carriers of `signal 1' and `signal 2', two additional aspects of DC biology are critical for the effectiveness of DC vaccines as a therapy for cancer; the induction of T-cells with the desirable effector functions and the ability to enter tumor tissues (that is the efficient delivery of `signal 3' and `signal 4') (Figure 2), and the preferential enhancement of the effector arm of immune responses without Treg hyperactivation. The latest advances in the area of DC biology should help in the development of vaccines able to induce T-cells with such properties, thus helping to bridge the gap between the effectiveness of therapeutic and protective vaccines.

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•• of outstanding interest

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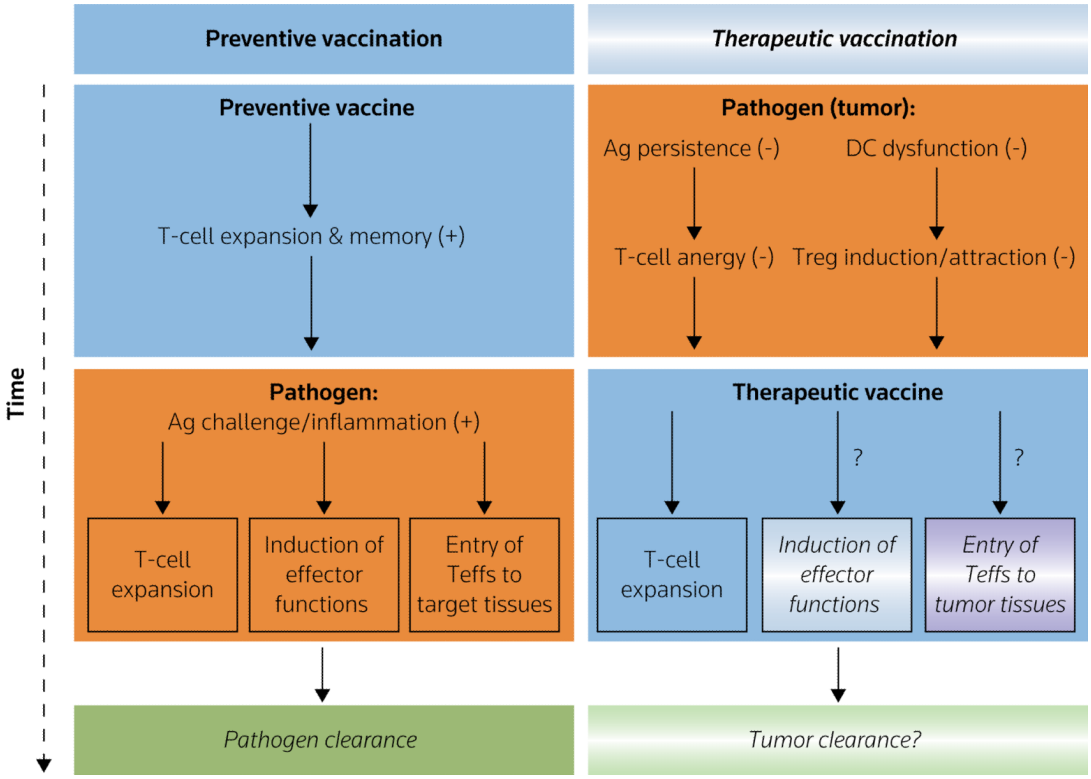

Figure 1. Therapeutic versus protective vaccines

In contrast to recall responses to tissue-invading microorganisms, T-cells in therapeutically vaccinated patients with cancer are not exposed to proinflammatory alarm signals (typically present in infected tissues), thus introducing the need for therapeutic vaccines, or additional components of immunotherapy, to induce the acquisition of tumoricidal effector functions and tumor-relevant homing potential. Additional problems in patients with cancer, who are persistently exposed to tumor-related antigens and immunosuppressive factors, include the dysfunction of endogenous dendritic cells (**DCs**) and other types of APCs that exhibit reduced ability to stimulate effector T-cells [30–43], as well as the hyperactivation of regulatory T-cells (**Tregs**) able to suppress active immunity [24–27]. Therefore, the effectiveness of therapeutic vaccines in such individuals is likely to require the presence of a fully mature DC population, resistant to suppression and able to either avoid or resist the interaction with immunosuppressive Tregs. Orange: pathogen-dependent events; blue: vaccination-dependent events; gradient: incomplete or only partial effectiveness, (+): positive/desirable effect; (−) suppressive/undesirable effect.

Ag antigen, **Teffs** effector T-cells

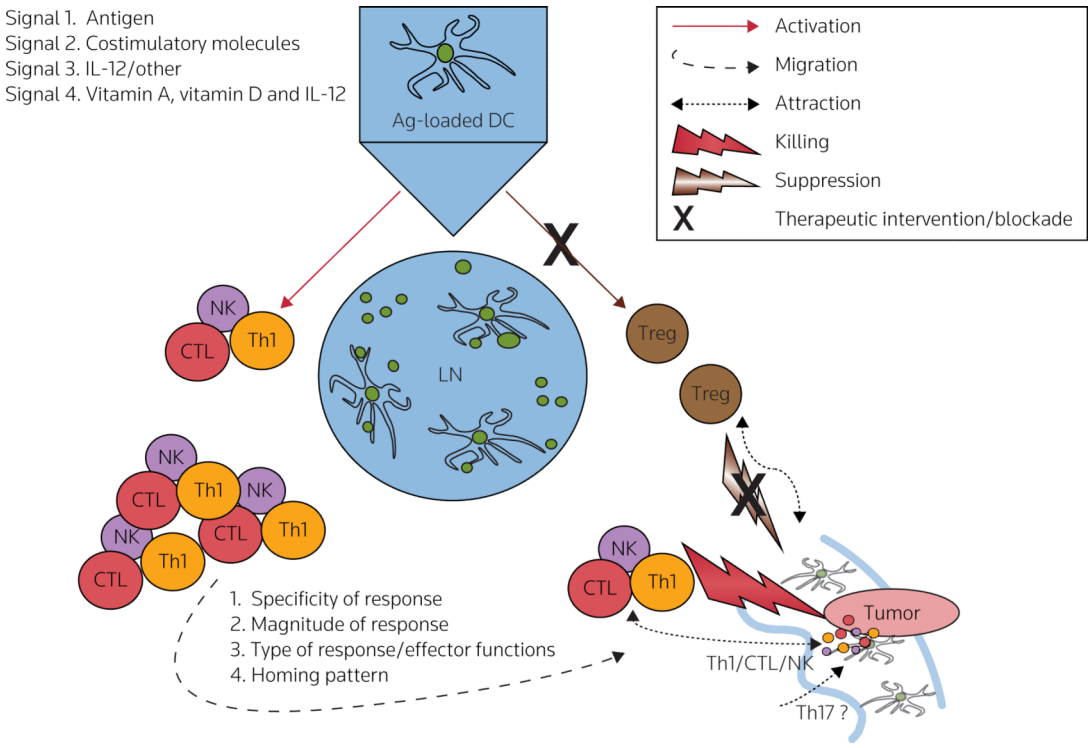

Figure 2. Roles of signals 1, 2, 3 and 4 in the induction of effective anticancer responses by DCs

Dendritic cells (**DCs**) provide T-cells with an antigenic 'signal 1' and a costimulatory 'signal 2' [55–57], both of which are required for the activation and expansion of pathogen-specific T-cells. DCs also provide an additional, polarizing 'signal 3', driving the development of immune responses towards type-1 or type-2 immunity [51], and leading to differential activation of particular effector mechanisms, as well as different capabilities in inducing cancer rejection [51,55–64]. There are data indicating that DCs may also provide T-cells with an additional signal (tentatively termed 'signal 4'), which regulates organ-specific trafficking of immune cells [123–126,129,139,140]. The key role of DCs in regulating the expansion and acquisition of effector functions and/or tumor-relevant homing properties has suggested the possibility of exploiting these properties in the development of effective cancer immunotherapeutics, able to preferentially activate and expand the appropriate immune effector cells (T-helper-1 cells [**Th1**], CTLs, NK cells, and potentially Th17 cells), while avoiding activation of regulatory T-cells (**Tregs**), the undesirable phenomenon observed with current cancer vaccines [27]. Moreover, the effectiveness of therapeutic vaccines is likely to benefit from the development of strategies aimed at selective recruitment of effector T-cells into tumors, such as tumor-specific modulation of chemokine production. Red: effector cell-mediated immunity, brown: regulatory/suppressor cell-mediated immunity.

Ag antigen, **LN** lymph node