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SYMPOSIUM IN WRITING

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Dendritic cells presenting tumor antigen

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Abstract Since the first identification of dendritic cells by Steinman and Cohn in 1973, progress in understanding their biology has included the development of novel methods of cell culture, recognition of critical aspects of migration and maturation, and appreciation of their major role as antigen-presenting cells (APC), and how this activity is regulated by cytokines and expression of accessory molecules. Dendritic cells are the major APC involved in the initiation of the immune response and the development of tolerance. There is considerable evidence that they can acquire antigen in the peripheral tissues and process, transport, and present it to T cells in secondary lymphoid tissue. A number of studies show that, in vitro or in vivo, antigen-pulsed dendritic cells can directly sensitize T cells and stimulate the development of antigen-specific immune responses, including both protective and therapeutic antitumor responses. In this paper, several important aspects of dendritic cell biology are discussed and a number of studies confirming the role of these professional APC in antitumor immunity are reviewed.

Key words Dendritic cells · Langerhans cells · Anticancer immunity · Biological therapy · Tumor immunology

Introduction

The absence of curative therapy for many forms of cancer and the grave prognosis of patients who fail conventional cancer treatment justify the application of novel, experimental therapies. One alternative to widely used chemotherapy and radiation treatments is to utilize the ability of the immune system specifically to target and eliminate tumor cells on the basis of expression of specific markers on their surface (TAA, tumor-associated antigens). The first step in developing effective antitumor therapies is to identify the means by which an immune response against tumor may be induced. Theoretically, after exposure to immunogenic peptides presented in the context of the proper MHC molecule, T cells are activated and expand clonally. During the past few years, several TAA have been identified, and other possible candidate molecules that may serve as target determinants have been proposed [54, 69]. Early attempts to use lymphokine-activated killer cells and tumor-infiltrating lymphocytes to transfer antitumor immunity in cancer patients revealed the existence of both theoretical and methodological obstacles and they have been of only marginal therapeutic efficacy [35].

Active immunotherapy using irradiated autologous or allogeneic tumor cells, or tumor cell products admixed with immunological adjuvants, has also been attempted for several decades. Although occasional evidence of the stimulation of antitumor activity of the immune system has been reported, vaccination using these approaches has not generally been very successful [6, 54]. Tumor cells could be modified as described in preclinical animal models to facilitate adhesion, antigen recognition, and co-stimulation and to improve the capacity of the malignant cells to serve as immunogens [23], but it is still unclear whether autologous tumor cells themselves need to be used as vaccines. Interestingly, the co-stimulatory signals delivered by the B7 family of molecules, which regulate the T cell response, have been recently demonstrated to have a crucial role in the initiation of antitumor response [12, 68]. Alternatively, tumor-specific peptide antigen could be presented to T cells by professional antigen-presenting (APC) cells bearing such co-stimulatory molecules, which might generate a more efficient and effective antitumor response or break an operational state of tumor tolerance. Thus, a critical target of vaccines is the specialized APC, the most immunologically potent of which are dendritic cells (DC) [3, 62, 25]. In fact, it has been recently demonstrated

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that tumor antigens are presented to T cells not by the tumor cells themselves but by host bone-marrow-derived APC [26], suggesting a potential approach for designing vaccination and therapy protocols.

Dendritic cells

DC are the heterogeneous professional APC that are critical for the initiation of T cell responses in vivo including sensitization of MHC-restricted T cells, development of T-cell-dependent antibody production and induction of immunological tolerance [61, 63]. The heterogeneity of these cells was emphasized by the identification of several different types, including Langerhans cells, interstitial DC (in heart, kidney, gut, lung), interdigitating DC, follicular DC, lymphoid DC (murine $CD8^+$ DC), and veiled DC from blood and lymph nodes. Morphologically, mature DC are large cells with elongated, stellated processes found in low numbers in lymphoid and non-lymphoid organs, as well as in the circulation. They typically lack cell-surface markers for B, T (TCR/CD3), natural killer (NK), or monocyte/macrophage cell lineages [19, 61], but express high levels of MHC class I and II, B7-1 (CD80), B7-2 (CD86), CD11a,b,c, CD40, ICAM-1 (CD54), and LFA-3 (CD58) molecules [1, 64]. Significantly, murine DC, including LC, splenic DC and cultured DC, express a functional common cytokine receptor γ chain, which may mediate cytokine-dependent regulation of their function [44].

DC originate from $CD34^+$ pluripotent hematopoietic progenitor cells in the bone marrow and migrate as immature cells to nonlymphoid tissue such as the skin, mucosa, and tumor [5, 26, 31]. Activation and subsequent migration of DC from non-lymphoid tissue to regional lymph nodes have been demonstrated to be early steps occurring during inflammatory reactions [38] and an important step in the development of a cell-mediated immune response against a number of pathogens [29, 45]. During antigen-induced immune responses, DC take up antigen, migrate through the afferent lymphatic system or the bloodstream to the lymphoid organs, and present the antigen to T cells. Thus, DC function involves three components that occur in sequence: a presentation step during which antigen is acquired, a processing step within which antigen undergoes proteolytic cleavage, and a sensitization step during which DC acquire the capacity to induce a response in T lymphocytes.

Dendritic cells and antigen presentation

Acquisition of antigen

Although early studies have mentioned that DC appear totally incapable of binding and internalizing endocytic markers, to date a large body of evidence suggests that the capacity to internalize and process antigen is a constitutive property of DC present in non-lymphoid organs [53] and that DC are as endocytically active as other APC [33]. It has been shown that, after skin painting with fluorescein isothiocyanate or s.c. in injection of antigen, DC in the draining lymph nodes express antigen and can stimulate sensitized T cells [8, 36]. In addition, following i.v. injection of antigen, DC are the only cells in the spleen to contain immunogenic fragments [14]. Similarly, oral antigen has been shown to be acquired efficiently by intestinal DC and such DC after migration to the lymph nodes can prime naive T cells in vivo [34].

Several distinct mechanisms for antigen capture have been described for DC. The first is a high level of fluid-phase uptake via constitutive macropinocytosis, allowing uptake of a high level of soluble antigens. The second is an uptake of antigens via mannose receptors, which are expressed on DC at high levels [56]. In particular, uptake of the yeast cell wall derivative, zymosan, is mediated by a mannose/β-glycan receptor [52]. Phagocytosis can be considered as a third mechanism of antigen capture by DC, since it has been reported that cultured bone-marrow-derived DC possess a high phagocytic potential with antigen non-covalently conjugated to polystyrene beads [57] as well as with BCG (Calmette-Guérin bacillus) [29], intact *Saccharomyces cerevisiae*, *Staphylococcus aureus*, *Corynebacterium parvum* [3], and *Salmonella* [42]. Furthermore, antigen-presentation experiments, in which soluble antigen and particle-absorbed antigen were given to DC, showed a more efficient presentation of antigen by DC that had taken protein conjugated with microbeads than by DC given soluble protein. In addition, antigen encountered by DC via the phagocytic route not only significantly increased the antigen-presenting activity, but also appeared to affect the development of the immune reaction directly by up-regulating interleukin-1α and interleukin-12 p40/p35 mRNA in DC [57].

Processing of antigen

After having been internalized, antigen is transported to an acidic endosomal compartment and degraded, and the derived peptides bind to the newly synthesized MHC class II molecules in the MHC-class-II-enriched compartments. Cultured DC are characterized by the presence of a large intracellular compartment containing class II molecules, cathepsin D, and lysosome-associated membrane protein 1, and are rapidly accessible to endocytic markers [56]. Furthermore, both mouse and human DC have been shown to produce and express significant amounts of MHC class II molecules. For instance, mouse splenic DC, when compared to B cells, express eight times the amount of MHC class II [48]. Interestingly, cultured human peripheral blood DC display a denser MHC class II expression on the plasma membrane and intracellularly than do freshly isolated DC [48]. It has also been shown that DC bearing antigenic peptide are able to prime MHC-class-I-restricted $CD8^+$ T cells in vivo [65]. Thus, the peptide-MHC complex may be expressed in high density on the cell surface as the DC differentiate.

Presentation of antigen

There is a general belief that DC undergo two stages of maturation [61]. According to this scheme, immature DC in non-lymphoid tissues take up antigen, process it and migrate to the regional lymph nodes. Tumor necrosis factor α is the principal cytokine implicated in the regulation of the migratory capacity of DC [15] as well as the efficient presentation of antigen by the DC [56]. Upon arrival, mature DC lose antigen-capturing and -processing properties and increase their T-cell-stimulatory capacity. It is important to note here that DC express high levels of adhesion molecules and counter-receptors, including CD11a (LFA-1), CD29 (β_1-integrin), CD54 (ICAM-1), CD58 (LFA-3), VCAM-1, VLA-1,4,5,6 (CD49a,d,c,f), and CD44 [1]. The presence of adhesins explains the capacity of DC to form clusters with a large number of T lymphocytes for prolonged periods [18]. Presumably, expression of the adhesion molecules permits mature DC to facilitate T cell recognition of specific complexes between peptides and MHC class I or class II molecules expressed on their surface. In fact, the high efficiency of DC in T cell activation has been found to correlate well with up-regulation of adhesion and co-stimulatory molecules as well as with enhanced expression of MHC class II molecules [22]. Furthermore, DC significantly augment production of interferon γ by cultured concanavalin-A-stimulated naive lymph node cells [24]. Because DC stimulate T lymphocytes isolated from non-immunized animals significantly more efficiently than do macrophages or B cells [9, 22], the hypothesis that DC are the initiating APC in the development of the immune response has been widely accepted [61, 67].

Thus, DC are as active as other APC with regard to the capture, processing and presentation of different antigens and can be considered as key cells in presenting allo-, viral or synthetic antigens to class-I-restricted CTL as well as to class-II-restricted $CD4^+$ T cells. A number of observations have confirmed this conclusion. Both a primary anti-viral proliferative T-cell response and virus-specific CTL can be induced by stimulating unprimed splenocytes with DC infected with influenza virus [37]. Likewise, lymph-node-derived DC, either pulsed with antigen in vitro or obtained from skin-painted mice, can present chemical allergens to naive lymphocytes in vitro and induce their proliferation [32, 36]. Similarly, MHC-class-II-restricted T lymphocytes can be elicited by footpad immunization with antigen-pulsed DC capable of presenting processed antigen for several days [28]. Interestingly, DC pulsed with haptenated monoclonal antibody to MHC class II have been shown to induce a rapid primary humoral anti-hapten response, whereas DC pulsed with control conjugates, i. e. haptenated non-binding monoclonal antibody, give only weak responses [43].

In summary, data discussed above allow us to conclude that DC can be used as a physiological adjuvant in vivo to activate MHC-restricted, antigen-specific T cells as well as to induce T-cell-dependent humoral responses. In fact, DC, pulsed with peptide [65] or protein [47] and inoculated into mice, induce strong CTL responses in vivo. Similarly, it has been shown that DC are superior to other cells in the presentation of Sendai virus to cytotoxic T lymphocyte precursors [30]. Of particular interest is the finding that bone marrow DC pulsed with ovalbumin peptide are potent inducers of ovalbumin-specific CTL responses in vivo, compared with splenocytes pulsed with this peptide, or compared with immunization with free peptide mixed with adjuvant [50]. In addition, the important role of bone-marrow-derived APC in presenting MHC-class-I-restricted tumor antigens has recently been demonstrated [26]. Likewise, CTL activation in vivo has also been observed after vaccination with bone marrow DC loaded with soluble TAA [49]. These results provide the basis for exploring the role of DC in the MHC-class-I-restricted immune response and suggest that presentation of TAA by DC may be a promising new strategy for using DC in cytotoxic-T-lymphocyte-mediated immunotherapy of cancer.

Dendritic cells and antitumor immunity

Since DC have been shown to present tumor antigen, and to initiate an effective immune response, the question arises whether DC can be used to induce antitumor immunity in vivo. Clinical studies have demonstrated changes in Langerhans cell number and morphology in the vicinity of epithelial malignancies [66] and suggest a correlation between the amount of tumor-associated DC and clinical prognosis for a number of tumor types [4]. Thus, it appears that DC may play an important role in cancer development and that they can be used as a tool to stimulate antitumor immunity in vivo.

In vivo protection studies

Epidermal Langerhans cells, pulsed in vitro with tumor fragments derived from S1509a fibrosarcoma and innoculated subcutaneously, conferred tumor-specific protective immunity in naive animals [21]. It has been reported that immunization with ovalbumin-peptide-pulsed epidermis-derived DC or Langerhans cells induces protective immunity to the MO5 ovalbumin-peptide-transfected melanoma [10]. Yan et al. [70] have shown that dermal APC are also capable of presenting TAA and initiating a strong protective antitumor immune response in the S1509a spindle-cell tumor system. Similar protective immunity has been demonstrated using tumor-pulsed splenic APC [58] and bone marrow DC in the murine KLN205 carcinoma and B16F10 melanoma [39, 40]. For instance, immunization with bone marrow DC loaded with soluble TAA protected 60% of mice challenged with live tumor cells, while mice receiving in addition a soluble TAA boost 5 days after the priming with pulsed DC were completely protected against tumor challenge [49]. It has also been shown, using a murine B cell lymphoma model, that a tumor-specific (anti-idiotype)

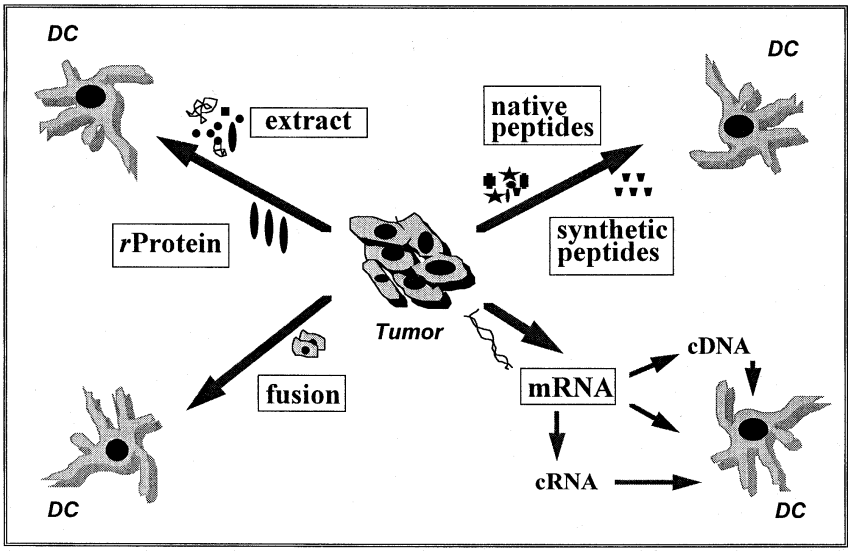

Fig. 1 Four different sources of tumor antigens that can be used for the creation of dendritic-cell-(DC)-based vaccines. These include: (i) RNA and/or DNA encoding a specific sequence of a tumor-associated antigen, (ii) peptides, natural (stripped) or synthetic, restricted for MHC class I and specific for the malignant cells, (iii) protein extract or protein, needed to be acquired and precessed by antigen-presenting cells, and (iv) fusion with tumor cells

response can be induced by immunization with DC loaded in vitro with idiotype protein, which protects the mice against a subsequent challenge with a lethal dose of tumor cells [17].

DC are also important in inducing secondary antitumor T-cell responses, as they efficiently present antigen to previously primed (memory) T cells [27]. Cohen et al. [13] have demonstrated that DC have the capacity to present TAA, derived from MCA-induced mouse fibrosarcomas, to primed T cells. The mechanism of protective immunity may involve both the cellular and humoral pathways. In fact, injection of antigen-pulsed DC also induced a rapid primary humoral anti-hapten response [43], including a response to TAA [60], which suggests that dendritic APC may potentiate antibody-dependent cytotoxicity against tumor cells in vivo [46]. Porgador et al. [51] have shown that $CD4^+$ T cells are required for the induction of an antitumor, $CD8^+$ T-cell-dependent immunity by DC, but do not participate in the effector phase. This conclusion is in agreement with a recent report showing that the antitumor effect of peptide-pulsed DC is also mediated by their ability to provide co-stimulation, since inoculation of the chimeric fusion protein CTLA4-Ig virtually abrogated the effect of DC [71]. Interestingly, Chaux et al. [11] have reported that CTLA4-Ig significantly delays and occasionally suppresses REGb (colon adenocarcinoma) rejection in rats, and that the immune response leading to REGb tumor rejection is initiated by $B7^+$ APC interacting with T lymphocytes.

In vivo tumor-regression studies

There are only a few studies that have evaluated the therapeutic efficacy of TAA-loaded DC in animals or patients with established tumors. In two recent publications it has been shown, using MCA205, TS/A, C3, and 3LL mouse tumor models, that multiple administration of bone marrow DC pulsed with unfractionated or synthetic TAA results in rejection or marked suppression of the growth of established tumors [41, 71]. Similarly, Gabrilovich et al. [20] have reported that immunization with p53-peptide-pulsed bone-marrow DC prolongs the survival of mice with established tumors expressing a mutant human *p53* gene. These data support the potential clinical application of TAA-pulsed DC as a novel human cancer therapy. Since a number of TAA have been identified for human tumors, such as melanoma and breast cancer, pulsed DC can represent a promising cell-based vaccine for cancer clinics, especially when a large number of DC can be generated from human $CD34^+$ precursor from the peripheral blood. In fact, Siena et al. [59] have described an efficient ex vivo generation of DC from blood cell transplantants in cancer patient. These DC were utilizable for tumor vaccination, since they were powerful stimulators of (i) allogeneic T cell proliferation in mixed lymphocyte reactions, (ii) autologous HLA-DR-restricted $CD4^+$ T cell proliferation in response to presentation of antigens, and, what is most important, (iii) HLA-A2-restricted $CD8^+$ CTL activation in response to presentation of Melan-A/MART-1 melanoma synthetic peptide [59]. It is important to mention a pilot study of Hsu et al. [25] concerning vaccination of patients with follicular B cell lymphoma, using autologous antigen-pulsed DC. All patients developed measurable antitumor cellular immune responses, and clinical response was seen in several patients. Thus, the stimulation of antitumor immunity by the administration of DC with TAA coding for signals important for the induction of a potent immune response appears to be a promising new addition to the wide spectrum of cancer-specific immunotherapies or vaccinations.

Together, these data demonstrate that DC are capable of inducing sensitization against TAA, as well as presenting TAA to primed tumor-specific T lymphocytes and inducing an effective CTL response.

Conclusion

In summary, the data discussed above, clearly suggest that DC pulsed with tumor antigen in vitro and administered to animals initiate an efficient antitumor immune response. Such immunity protects animals against inoculation with tumor cells and, in some cases, causes suppression of growth or regression of established tumor. The increasing success of this approach will be dependent on a full understanding of antigen handling. Pulsing of DC with TAA is a common strategy for the loading of tumor antigen into DC (see Fig. 1). This includes presentation of natural stripped tumor-cell-surface peptides, synthetic tumor peptides or proteins, or extract (lysate) of tumor cells followed by different steps of purification and separation. Another strategy for the presentation of TAA by DC is the establishment of immunogenic DC/tumor-cell chimeras by fusion with tumor cells. Among promising strategies of DC loading with the correct antigen is the transduction of genes encoding a relevant protein into DC. Interestingly, Alijagic et al. [2] have reported that the transfection of human DC, obtained from blood precursors in granulocyte/macrophage-colony-stimulating factor/interleukin-4 cultures, with human tyrosinase leads to protein synthesis and presentation of antigenic peptide in the context of MHC molecules on the cell surface of DC, as demonstrated by clustering and release of tumor necrosis factor by a specific CTL clone. In addition, it is possible that so-called genetic immunization, which has been shown to be able to induce protective tumor immunity [16] is mediated, at least in part, by DC. In fact, Bouloc et al. [7] reported that Langerhans cells effectively present antigen after i. d. injection of DNA encoding this antigen and initiate primary as well as secondary T cell responses. Clinical trials are already in progress to test many of the notions discussed here and the efficacy of these approaches.

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