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Dendritic Cells: Immunobiology and Cancer Immunotherapy

Meeting Report

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A recent workshop on "Dendritic Cells: Biology and Therapeutic Applications," sponsored by the Juan March Foundation, brought together basic and clinical research scientists to discuss the mechanisms underlying the control of immune responses and tolerance by dendritic cells (DCs), as well as recent research in cancer immunotherapy based on DC vaccination. Particular emphasis was placed on antigen processing and presentation by DCs, C-type lectin antigen receptors, DC maturation and polarization of T cell responses, the control of immunity versus tolerance by DCs, the developmental origin of DCs, and the use of DCs in cancer immunotherapy.

The past decade has been marked by spectacular progress toward understanding how dendritic cells (DCs) control the immune system, arising from intensive work dealing with a large diversity of DC research fields, as summarized in Figure 1. These include the description of the mechanisms underlying the processing and presentation of antigens derived from pathogens, apoptotic and tumor cells, the organization of the DC-T cell immune synapse, the discovery of C-type lectin receptors for antigen capture, the control of DC activation by Toll-like receptors (TLRs), the relevance of plasmacytoid DCs (pDCs) in antiviral responses, the role of DCs in polarizing T helper cell responses, the regulation of natural killer (NK) and B cell responses, the in vivo analysis of DC traffic and immune interactions, the control of immunity versus tolerance by DCs, the role of DCs in microbial infections, autoimmune disorders, allergic reactions, graft rejection, and antitumor immune responses, and the differentiation pathways of DCs. These advances in DC immunobiology, together with the improvement of DC transfection and in vitro differentiation techniques, have allowed the development of promising DC-based vaccination and cancer immunotherapy protocols, and other in vivo approaches.

Conscious of the importance of DCs in the control of immunity and of the therapeutic potential of DC vaccination, the Juan March Foundation sponsored a workshop on "Dendritic Cells: Biology and Therapeutic Applications," which was organized by Ralph Steinman (Rockefeller University, New York, USA), Ignacio Melero (Universidad de Navarra, Pamplona, Spain), and Angel Corbí (Centro de Investigaciones Biológicas, Madrid, Spain) and took place in Madrid, October 6–8, 2003.

DC Immunobiology

DCs are generated from either myeloid or lymphoid bone marrow progenitors through intermediate DC precursors that home to sites of potential antigen entry, where they differentiate locally into immature DCs. After antigen capture in the presence of maturation signals associated with inflammation or infection, immature DCs are activated by TLRs, interferons (IFNs), or members of TNF-R family and undergo a complex maturation process. In vivo this process is paralleled by migration of DCs to T cell-rich areas of lymphoid organs, where they present antigen-derived peptides to antigen-specific T cells and direct their differentiation into T effector or memory cells. In addition, mature DCs can induce NK cell activation and B cell differentiation into antibody-forming cells. In contrast, antigen capture in the absence of activation stimuli may lead to the induction of T cell tolerance, as a result of antigen presentation by immature DCs in the absence of costimulation. An integrated view of DC immunobiology is shown in Figure 2.

Mechanisms of Antigen Processing and Presentation by DCs

Rolf Zinkernagel (Zurich, Switzerland) gave the opening presentation in this meeting. He presented experiments examining crosspresentation and crosspriming by tumor cells expressing various viral antigens. No crosspriming was detectable in vivo, nor was protection via such a mechanism demonstrable. Recent experiments revealed that poliovirus prime CTLs in normal C57B/6 mice and that polioviral antigens are translated in receptor-negative mice. Therefore, the original assumption of a need for crosspriming in immune responses against poliovirus in mice was questioned (Freigang et al., 2003). Zinkernagel concluded that while crosspriming cannot be absolutely excluded, its efficiency is at least a 1000 to 10,000 times less than direct presentation on MHC class I.

However, crosspresentation may be much more efficient for certain particulates. Sebastian Amigorena (Paris, France) presented a recent study of the pathways involved in DC crosspresentation of such antigens. He showed that newly formed phagosomes in DCs rapidly fuse with elements of the endoplasmic reticulum, thereby bringing the MHC class I loading machinery into the endocytic compartment. These phagosomes can then function as self-contained MHC class I loading compart-

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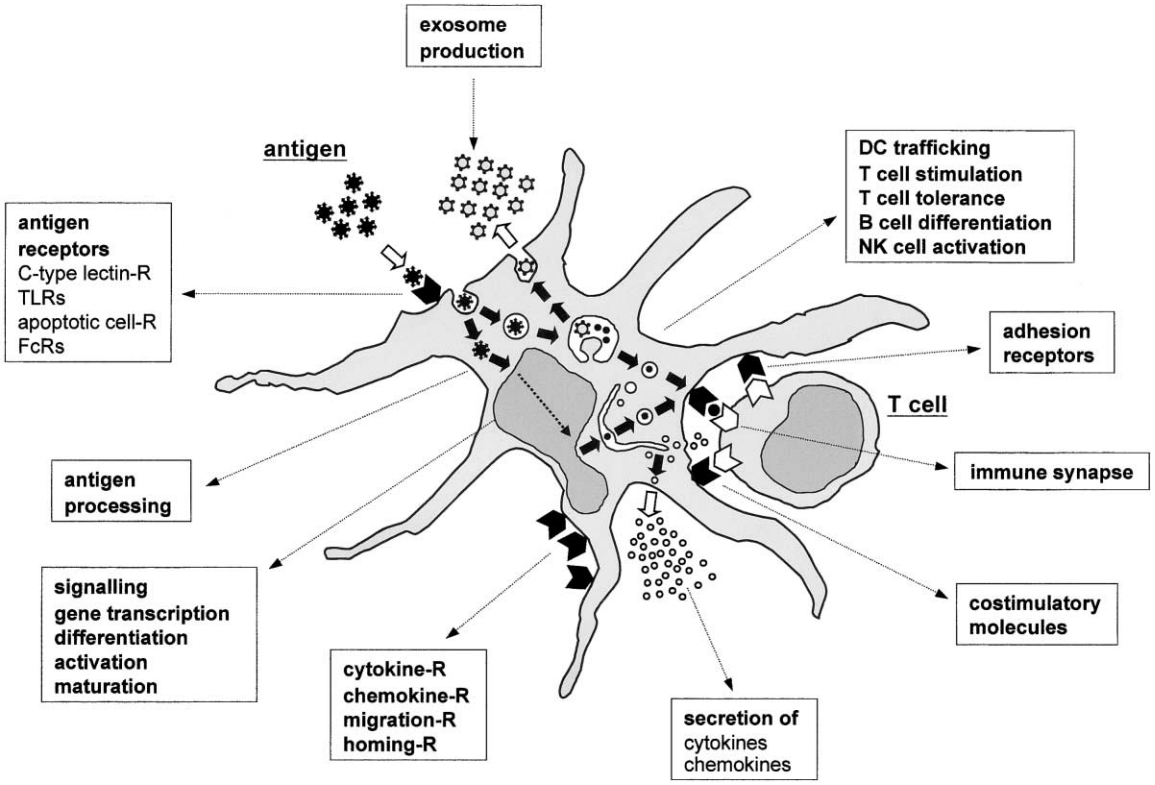

Figure 1. Diagram Summarizing the Main Current Research Topics Related to DCs

ments, allowing any phagosomal antigens escaping to the cytosol to be rapidly processed by proteasomes and reimported via TAP (Guermonprez et al., 2003). Thus, phagosomal loading favors crosspresentation by diminishing competition between exogenous antigens and endogenous antigens, which are loaded in the endoplasmic reticulum.

Evelina Gatti (Marseille, France) presented a recent study about the regulation of the degradation of defective ribosomal products (DRiPs) in DCs. DRiPs, which account for almost 30% of newly synthesized proteins, represent the main source of MHC class I-restricted peptides. At the onset of maturation DCs specifically aggregate ubiquitinated DRiPs and form *d*endritic cell *a*ggresome-*l*ike *i*nducible *s*tructures (DALIS) (Lelouard et al., 2002). These structures accumulate transiently in the cell and delay the degradation of DRiPS, although proteasome function itself is not affected. Accumulation of DRiPs is likely to influence MHC class I presentation. In agreement with this hypothesis, she showed that at the onset of maturation MHC class I-peptide complexes are rapidly and transiently downregulated and that the kinetics of this downregulation correlates with the formation of DALIS.

Francisco Sánchez Madrid (Madrid, Spain) discussed recent findings on the dynamics of initial exploratory interactions between T lymphocytes and DCs (Mittelbrunn et al., 2002; Montoya et al., 2002). Live time-lapse confocal microscopy analyses of GFP adhesion molecules transfected in T cells and DCs revealed that ICAM-1, ICAM-3, and LFA-1 are involved in the early adhesive T-DC contacts at both T and DC sides.

Maria Mittelbrunn (Madrid, Spain) showed that in the presence of antigen, the β1 integrin VLA-4 is recruited to the periphery of the immune synapse, colocalizing with LFA-1. She further addressed the role of VLA-4 in T cell activation by showing that VLA-4 engagement regulates Th1 responses both in vitro and in vivo. Finally, she reported a protective effect of treatment with anti-VLA-4 antibodies in a model of autoimmune nephritis by balancing Th responses.

C-Type Lectin Antigen Receptors

C-type lectins expressed on immature DCs represent an important class of antigen receptors, which recognize glycosylated structures (reviewed in Figdor et al., 2002). C-type lectins may not only function as receptors mediating the internalization and processing of pathogens to enhance antigen presentation by DCs, but could also mediate cell adhesion and signaling.

The latter was highlighted by Yvette van Kooyk (Amsterdam, The Netherlands) who discussed a novel high-affinity cellular interaction between granulocytes and DCs. This involves the recognition of nonsialylated Lewis^x expressed by the neutrophil receptors CD11b and CD66a by the C-type lectin DC-SIGN expressed on DCs. She further reminded us that DC-SIGN seems to be targeted by many different pathogens (viruses, bacteria, yeast, and parasites) to escape immunity by subverting intracellular trafficking or interfering with DC activation (van Kooyk and Geijtenbeek, 2003). For example, DC-SIGN mediates HIV internalization, but viral particles stay in early endosomes and are transported again to the cell surface, allowing HIV to mediate T cell infection

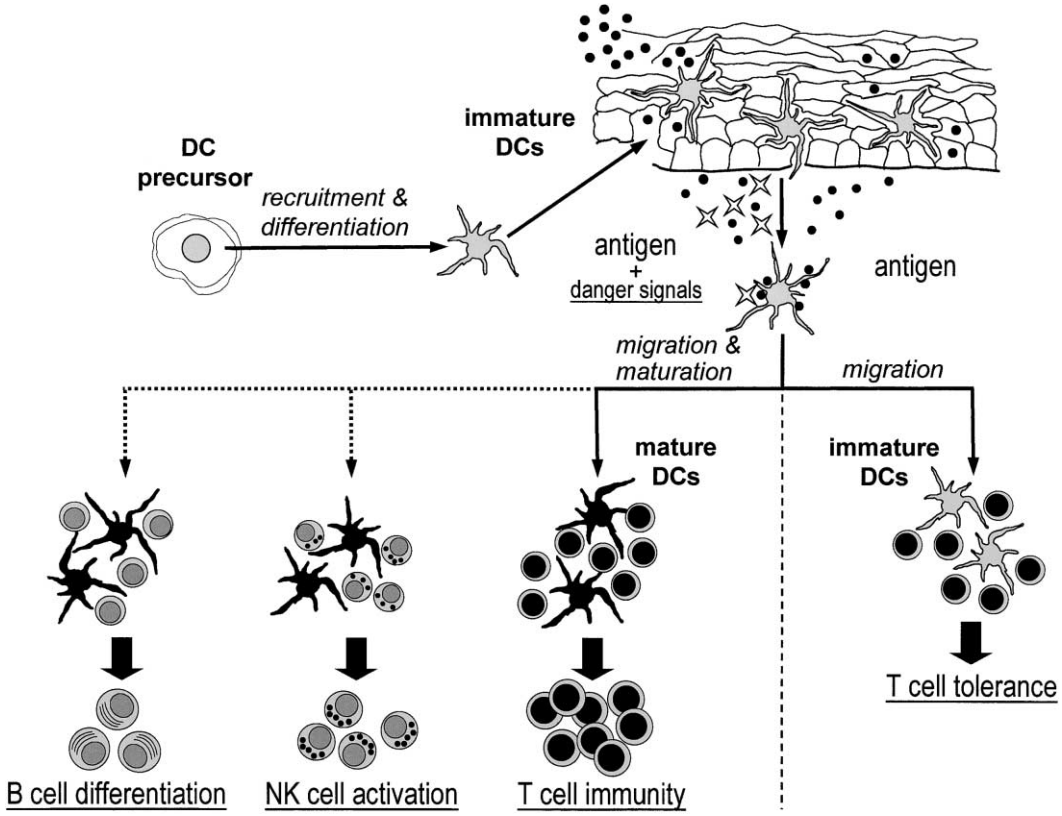

Figure 2. Integrated View of DC Immunobiology

in *trans*. Similarly, ManLAM from *Mycobacterium tuberculosis* binds to DC-SIGN and alters DC maturation by interfering with TLR signaling (Geijtenbeek et al., 2003). This suggests that DC-SIGN could have a primary function in recognizing self-antigen and maintaining self-tolerance, which is exploited by pathogens to suppress immune activation.

The involvement of DC-SIGN in pathogen recognition was further highlighted by Angel Corbí (Madrid, Spain) who addressed how DC-SIGN mediates the interaction of DCs with clinically relevant parasite and fungal pathogens, and how the level of expression of DC-SIGN differentially affects its adhesion and pathogen recognition abilities. He further showed that DC-SIGN engagement on the cell surface triggers intracellular signals, which could potentially affect the DC maturation process. However, it remains unclear whether this signaling signified that DC-SIGN functions as an actual pattern-recognition receptor, leading to DC activation, or modulates TLR-initiated signals. Other C-type lectins are likely to also bind pathogens, but pinpointing their carbohydrate ligands remains a challenging area of cell biology, as oligosaccharides cannot be readily cloned, each being the product of multiple glycosyltransferases.

Ten Feizi (London, UK) discussed a carbohydrate microarray system for generating large repertoires of immobilized oligosaccharide probes, which is proving to be a powerful means both of detecting protein-carbohydrate interactions and assigning the sequences recognized (reviewed in Feizi et al., 2003). She described the application of this technology to a recombinant soluble form of murine SIGN-R1, which, in conjunction with binding experiments with SIGN-R1-transfected cells, has revealed that high-affinity ligands for this receptor are mannosyl-fucosyl glycans, whereas dextran is a low-affinity ligand.

Eirikur Saeland (Amsterdam, The Netherlands) expanded the topic of DC-expressed C-type lectins to a discussion of their possible function in recognition of tumor antigens, some of which, such as MUC1, can be aberrantly glycosylated. By studying the interaction of recombinant MUC1 with human DCs, he identified MGL (macrophage Gal/GalNAc specific C-type lectin) as a receptor for tumor-associated MUC1 and showed that binding was dependent on the particular glycoform in question. Further elucidation of MGL recognition could extend the potential of MUC1 as a target for immunotherapy.

DC Activation and Polarization of T Cell Responses

TLRs function as sensors of microbial infection and play a critical role in the induction of innate and adaptive immune responses (reviewed in Kopp and Medzhitov, 2003). TLR-mediated DC activation and maturation, which are dependent on NF-κB and MAP kinase signaling pathways, lead to upregulation of MHC and costimulatory molecules, as well as production of cytokines and other soluble factors. Together, these act to sustain the clonal expansion of newly activated T cells and their

differentiation into effectors to determine the polarization of T helper cell responses.

Caetano Reis e Sousa (London, UK) showed that DC maturation can be induced by TLR ligands but that it can also occur in response to signals from newly activated $CD4^+$ T cells independently of innate priming. T cell-driven DC maturation takes place both in *cis* and in *trans*, affecting all DCs in the microenvironment, including ones that do not bear specific antigen. In contrast, IL-12 production by DCs shows an absolute requirement for TLR signals and can only be amplified, but not initiated, by signals from newly activated $CD4^+$ T cells delivered in *cis* (Spörri and Reis e Sousa, 2003).

The role of TLR-mediated induction of costimulation and cytokines in DCs was further discussed by Ruslan Medzhitov (New Haven, USA) who showed that in addition to the upregulation of costimulation by DCs, TLRs induce a cytokine-dependent blockade of T regulatory (Treg) cell suppression, mediated by IL-6 (Pasare and Medzhitov, 2003). On the basis of the analysis of MyD88-deficient mice, he concluded that TLR-induced cytokine production and block of Treg cells was MyD88 dependent both in vitro and in vivo. He finally emphasized the importance of TLRs in T cell activation by showing that no DC-induced T cell activation occurs in the absence of TLR ligands in response to "pure antigens," i.e., not containing TLR ligands. Thus, innate signals promote immunity by controlling multiple parameters of T cell priming by DCs.

The concept that polarization of T helper responses relies on specialized DC subsets was challenged by Anne O'Garra (London, UK). She reported that different DC subpopulations either differentiated in vitro or isolated from the spleen can induce Th1 or Th2 effector cells in vitro depending on the antigen dose and the activation stimulus. High antigen doses induced a Th1 response, while low doses induced a Th2 cytokine profile (Boonstra et al., 2003). On the other hand, the capacity of myeloid DCs versus pDCs to induce Th1 responses was shown to be dependent on activation with LPS and CpG, respectively, correlating with the high expression of TLR4 and TLR9 by myeloid DCs and pDCs.

Control of Immunity and Tolerance by DCs

Recent data have evidenced that, in addition to their role in the induction of effector T cell responses, DCs have an important role in peripheral tolerance. Induction of tolerance by DCs occurs in the steady state as a consequence of antigen presentation in the absence of inflammation or infection, and can be ascribed in different settings to T cell deletion, T cell anergy, or the induction of Treg cells (reviewed in Steinman et al., 2003).

Ralph Steinman (New York, USA) emphasized the potential of antigen targeting to DCs via antigen receptors for DC-mediated vaccination. By using an experimental system based on the injection of conjugates of ovalbumin (OVA) linked to antibodies against the C-type lectin DEC-205 (anti-DEC-OVA), he showed that in the steady state, OVA-loaded DCs via anti-DEC-OVA efficiently presented OVA to both MHC I- or MHC II-restricted T cells to induce peripheral tolerance (Bonifaz et al., 2002). In contrast, when anti-DEC-OVA was injected together with anti-CD40 or α-galactosylceramide, strong T cell expansion was induced (Fujii et al., 2003). Anti-DEC-OVA conjugates stayed for long periods after injection in the lymph nodes, inducing a prolonged and systemic antigen presentation to OVA-specific T cells. Antigen targeting via DEC-205 resulted in stronger effector T cell responses than those obtained with antigen-pulsed ex vivo-derived DCs or antigen in complete Freund's adjuvant. Finally, Steinman underscored the importance of DC maturation for the induction of effective T cell responses by showing that OVA-specific effector T cell development was significantly enhanced when antigen presentation was concurrent with influenza infection.

Michel Nussenweig (New York, USA) discussed recent data on the mechanisms controlling the induction of T cell tolerance by DCs using a model of experimental allergic encephalomyelitis (EAE), induced by treatment with the MOG peptide, derived from the myelin oligodendrocyte protein, in complete Freund's adjuvant. He reported that treatment with conjugates of anti-DEC-205 with the MOG peptide (DEC-MOG) prevented the development of EAE. Injection of anti-DEC-MOG determined the differentiation of nonanergic, unresponsive MOG-specific T cells, without Treg cell function.

Antonio Lanzavecchia (Bellinzona, Switzerland) emphasized the importance of "tissue conditioning" for DC migration by showing that CCR7-dependent DC migration to the lymph nodes was increased after a first injection of DCs or after treatment of the injection area with TNF-α or IL-1β, which correlated with the upregulation of the CCR7 ligand SLC by the lymphatic endothelium (MartIn-Fontecha et al., 2003). In the second part of his talk, he showed data supporting a model of progressive T cell differentiation, proposing that the signal strength resulting from the DC-T cell interaction determines the fate of the responding T cells (Gett et al., 2003). Low signal strength induced the proliferation of naive T cells but not the acquisition of effector function. At higher signal strength T cells acquire effector function and the capacity to migrate to inflamed tissues, while even higher signal strength induced T cell death. Finally, Lanzavecchia presented exciting data showing that activation of pDCs induced the upregulation of CXCR5 and the production of its ligand BCA1/CXCL13, suggesting a role for pDCs in mediating the formation of T cell-B cell interactions within B cell follicles.

The pivotal role of type I (α, β) and type II (γ) IFNs in the reciprocal activation of NK cells and DCs was addressed by Giorgio Trinchieri (Dardilly, France). He reported that IL-2-activated NK cells induced the maturation of monocyte-derived DCs by a mechanism requiring IFN-γ production by NK cells, as well as cell contact (Gerosa et al., 2002). IL-2-activated NK cells can also induce pDC maturation and production of IFN-α, which can be further increased in the presence of CpG. Conversely, when activated by virus, CpG or CD40L, pDCs acquire the capacity to activate resting NK cells by a cell contact-independent mechanism that can be blocked with anti-IFN-α antibodies. These data support a sequential model of NK-DC activation that proposes that activated pDCs secreting type I IFNs induce NK cell activation; activated NK cells then produce IFN-γ which induces the maturation of myeloid DCs and pDCs. Finally, Trinchieri underscored the role of NK cells in IFN-γ production after TLR8 engagement by the antiviral com-

pound R-848 (imidazoquinoline resiquimod), in a process requiring the participation of myeloid CD11c$^+$ DCs.

Lieping Chen (Rochester, USA) presented data on a new costimulatory pathway including B7-H1, B7-DC, PD-1, and possibly another costimulatory receptor (Wang et al., 2003). B7-H1 is a costimulatory molecule belonging to the category of dual function costimulators, which can display either stimulatory or inhibitory function. Several published studies analyzing mice deficient for PD-1 (programmed death-1), a ligand for both B7-H1 and B7-DC, support its inhibitory role since PD-1$^{-/-}$ mice develop autoimmune diseases. Expression of B7-H1 by tumor cells induces deletion of activated T cells, again supporting the inhibitory function of the B7-H1/PD-1 interaction. In contrast, treatment with blocking anti-B7-H1 monoclonal antibodies suppressed the development of chronic intestinal inflammation and reduced the production of IFN-γ, IL-2, and TNF-α by intestinal CD4$^+$ T cells (Kanai et al., 2003). Comparative molecular modeling followed by site-directed mutagenesis revealed B7-H1 mutants that do not bind PD-1 induce proliferation and IFN-γ production by T cells. These data suggest that B7-H1-mediated T cell activation involves the interaction of B7-H1 with an as yet unidentified costimulatory ligand for B7-H1.

The role of costimulatory signaling in DC-mediated T cell stimulation was further underlined by Tahiro Shin (Baltimore, USA) who addressed the function of a novel costimulatory molecule B7-DC, whose expression is restricted to DCs. B7-DC appears to synergize with B7-1 and B7-2 in the induction of CD4$^+$ T cell proliferation (Shin et al., 2003). Analysis of B7-DC-deficient mice highlighted the role of this molecule in the initiation of CD4$^+$ T cell responses, since proliferation of naive, but not activated, CD4$^+$ T cells was disminished in B7-DC$^{-/-}$ mice. Experiments performed with B7-DC$^{-/-}$ mice or involving a treatment with anti-B7-DC-blocking antibodies suggest a role for this molecule in the induction of Th1 responses.

Origin and Precursors of DCs

Carlos Ardavín (Madrid, Spain) discussed recent data derived mostly from in vivo DC reconstitution assays in the mouse showing that the same DC subpopulations, including conventional DCs and pDCs, can be generated from either myeloid or lymphoid progenitors (reviewed in Ardavín, 2003). These experiments do not support the existence of independent myeloid and lymphoid DC subpopulations as previously proposed but instead point to a DC differentiation model relying on a dual contribution of myeloid and lymphoid differentiation pathways. However, different experimental approaches suggest that in vivo pDCs derive from the lymphoid lineage. Carlos Ardavín also presented data suggesting that, during microbial infections, blood-borne DC precursors are recruited to the lymphoid organs, where they differentiate into fully competent DCs, including CD8α^- DCs, CD8α^+ DCs, and B220$^+$ pDCs, supporting the possibility that all DC subsets might derive from a single DC common precursor (Martínez del Hoyo et al., 2002).

María L. Toribio (Madrid, Spain) focused on the developmental origin of intrathymic DCs in humans and showed that CD11c$^+$ DCs resident in vivo in the human postnatal thymus are truly myeloid DCs, as they develop from CD34$^+$ early thymic progenitors through CD34^{lo} intermediates with upregulated GM-CSF receptor expression, which branch off the main intrathymic developmental pathway and lose the capacity to generate T cells, but display myelomonocytic potential (de Yebenes et al., 2002). She also showed that this decision point is influenced by Notch1, since overexpression of an activated form of this receptor, shown to affect intrathymic differentiation of human T cells (Garcia-Peydro et al., 2003), prevents early thymic progenitors from adopting a myeloid cell fate and simultaneously blocks development of DCs. These data challenge the current view that the thymus is colonized by a lymphoid-restricted progenitor and provide evidence that a more immature lymphomyeloid precursor population is actually seeding the human postnatal thymus.

Gwendalyn Randolph (New York, USA) underlined the differential migration properties of human monocyte subsets, defined on the basis of the expression of CD16 (FcγRIII). Using an in vitro system, she showed that CD16$^+$ were more effective than CD16$^-$ monocytes in reverse transmigration and differentiation into DCs (Randolph et al., 2002). Others have reported that CD16$^+$ and CD16$^-$ subsets share patterns of chemokine receptor expression with mouse Gr-1$^-$ and Gr-1$^+$ monocytes, respectively, which have been shown to display distinct trafficking and differentiation behavior. She showed that further similarities between CD16$^+$ human monocytes and F4/80$^+$ CD115$^+$ Gr-1$^-$ mouse monocytes included higher expression of costimulatory molecules than other monocytes. The Gr-1$^-$ subset of monocytes was found to be nearly absent in CCR8-deficient mouse blood, and CCR8$^{-/-}$ had defects in the differentiation of monocytes into DCs. Anti-CCR8 antibodies blocked human monocyte reverse transmigration in vitro. In conclusion, she suggested that both CD115$^+$ Gr-1$^-$ mouse monocytes and CD16$^+$ human monocytes readily develop into DCs via CCR8-mediated signals.

DC-Based Cancer Immunotherapy

In the last session of this meeting, several recent approaches aimed at optimizing the use of DCs in immunotherapy were reported. Attempts to induce effective immune responses were based on the use of DCs or tumor cells as vectors, in association with either the expression of recombinant cytokines or T cell costimulation molecules, or with monoclonal antibodies that boost immunity, either by stimulating DCs or T cells, or by blocking Treg cells (reviewed in Schuler et al., 2003).

Ralph Steinman presented results from a phase I clinical trial performed by the research group of Jacques Banchereau (Dallas, USA), in which CD34$^+$ precursors were differentiated into mature DCs, loaded with tumor antigen peptides, and injected into metastatic melanoma patients (Palucka et al., 2003). Effective antitumor immunity and objective clinical responses were observed in several patients. Interestingly, those patients that developed melanoma-specific CTLs survival significantly longer than those that did not mount melanoma-specific CTLs.

Eli Gilboa (Durham, USA) showed that in three phase

I/II clinical trials, the majority of patients treated with mRNA transfected DCs exhibited T cell responses against the mRNA-encoded antigens (Su et al., 2003). To improve the immunopotency of the transfected DCs he described a method for enhancing the class II-restricted presentation of endogenously expressed antigens by transiently inhibiting invariant chain expression using antisense oligonucleotides (Zhao et al., 2003). Mice immunized with antisense-treated mRNA transfected DCs stimulated a more potent and longer lasting $CD4^+$ and $CD8^+$ T cell response and exhibited enhanced antitumor immunity. He also showed that immunizing against angiogenesis-associated products (such as VEGFR-2, Tie-2, or VEGF) overexpressed in the tumor stroma inhibits tumor growth and argued that targeting stromal products could reduce the propensity of tumor cells escaping immune elimination (Nair et al., 2003).

Cornelis Melief (Leiden, The Netherlands) presented evidence that crosspresentation of tumor antigens to $CD8^+$ CTL occurs naturally for many tumors. This alone does not cause a tumoricidal $CD8^+$ CTL response. In many cases the suboptimal DC stimulation associated with tumor growth causes the $CD8^+$ T cells to remain stuck as "poised" T cells in the tumor-draining LN. This situation can be vastly exploited by DC stimulation through CD40 agonistic antibody or TLR ligands (CpG ODN, LPS), leading to a systemic tumoricidal $CD8^+$ effector CTL expansion associated with tumor eradication (van Mierlo et al., 2002, and unpublished data).

Ignacio Melero (Pamplona, Spain) presented results on the efficacy of the injection into mouse tumor nodules of DCs that had been engineered to produce IL-12 using adenoviral transfection (Melero et al., 1999). The efficacy of this therapy can be enhanced by repeating doses, using semiallogenic (MHC haploidentical) DCs and by synergistic combination with systemic administration of anti-CD137 immunostimulating monoclonal antibodies (Tirapu et al., 2003). A clinical trial with patients suffering colon, pancreatic, and primary liver carcinomas based on ultrasound-guided intratumoral injection of DCs expressing recombinant IL-12 is ongoing.

Mario Colombo (Milan, Italy) presented results aimed at optimizing antitumor vaccination. To create cell vaccines able to attract and activate DCs in vivo, tumor cells were transduced with genes encoding GM-CSF and either CD40 ligand or OX40L. The last combination was the most effective in curing experimental lung metastases in animal models. Alternatively, tumor cells were engineered to secrete recombinant hsp70 in the extracellular milieu, independently from cell death. The results suggest that hsp70 redirected to the secretory pathway is an effective carrier for tumor antigen-derived peptides. Intralesional injection of an adenovirus encoding CCL16 attracted DCs and induced inflammation. This treatment has proven efficacious if given before surgery for prevention of metastases in the 4T1 mammary carcinoma model (Gri et al., 2003).

Gerold Schuler (Erlangen, Germany) showed the results from two recent phase I/II clinical trials in advanced stage IV metastatic melanoma, using mature DCs loaded with MHC class I and II tumor antigen-derived peptides. Upon vaccination CD4 and CD8 antigen-specific T cell responses were detectable in blood by Elispot and in blood and tumor tissue by tetramer analysis. Extensive work-up of a previously performed trial showed that induced responses were polyclonal (Godelaine et al., 2003). Disease stabilization and regression of individual, but not of all, metastases was observed in a significant proportion. Overall survival increased from 11–15 months in previous trials to 24 months in the closed class I + II peptide trial (Schuler-Thurner et al., 2002) and will presumably be higher in the still ongoing second trial.

Concluding Remarks

Research on DCs continuously reveals novel regulatory capacities of this extremely plastic and versatile cell type, which plays a crucial role in the initiation and control of immunity and tolerance, although paradoxically, DCs can also be responsible for the induction of some pathogen-mediated and autoimmune diseases, allergic reactions, graft rejection, and failure of the immune system in the defense against tumor progression. Therefore, a better understanding of DC immunobiology will provide essential insights allowing the manipulation of the immune system through DCs and, consequently, the development of novel strategies for the treatment of microbial and parasitic infections, the control of graft rejection, and the improvement of DC-based immunotherapeutic protocols for autoimmunity, allergy, and cancer.

Acknowledgments

We apologize to the many participants whose work has not been discussed due to space limitations. We are grateful to the speakers at the meeting for critical comments and helpful suggestions, and to Ralph Steinman, Ignacio Melero, and Angel Corbí for contribution to the organization of the stimulating scientific sessions. We also thank the Juan March Institute for funding this workshop.

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