[Frontiers in Bioscience 14, 808-817, January 1, 2009]

The divergence and interplay between pDC and mDC in humans

Norimitsu Kadowaki

Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, 54 Shogoin Kawara-cho, Sakyo-ku, Kyoto 606-8507, Japan

TABLE OF CONTENTS

1. ABSTRACT

Induction of appropriate types of innate and adaptive immune responses depending on the nature of antigens is crucial to cope with a variety of pathogens and concurrently to avoid pathological reaction to self antigens. Recent studies have been elucidating that the diversity of immune responses is critically controlled by dendritic cells (DCs). Two DC subsets have been identified in humans: myeloid DCs (mDCs) and plasmacytoid DCs (pDCs). The DC subsets recognize different microbial pathogens by expressing distinct repertoires of Toll-like receptors, and induce different types of innate and adaptive immune responses depending on environmental factors. Particularly, pDC precursors produce vast amounts of type I interferons in response to viruses and thus play an important role in anti-viral immunity. In addition, type I interferons derived from pDCs are instrumental in activating mDCs. Elucidating cellular and molecular mechanisms by which functions of the two DC subsets are modulated will lead to understanding the pathogenesis of various immune-related diseases and to developing novel immunological therapies.

2. INTRODUCTION

The immune system has evolved to eliminate a variety of microbial pathogens and at the same time to avoid responding to self antigens and innocuous antigens. Elucidating how this demanding task is accomplished is the main theme of immunology. Recent studies have been revealing that dendritic cells (DCs) are deeply involved in the process of differential responses to different types of antigens(1).

During innate immune responses at the site of infection, immature DCs located in inflamed tissues incorporate pathogens, and become activated in response to pathogens themselves and proinflammatory cytokines. Thereafter, the activated (also called mature) DCs migrate to draining lymph nodes and stimulate naïve T cells to differentiate into functionally competent effector T cells. Importantly, such T cell responses, especially those of $CD4^+$ T helper (Th) cells, are heterogeneous; naïve $CD4^+$ T cells differentiate into effector Th cells that produce different combinations of cytokines. These divergent Th

cell responses, the prototypes of which are called Th1 and Th2 responses, induce different types of immune responses appropriate to eliminate given pathogens. On the other hand, naïve $CD4^+$ T cells are able to develop into immunosuppressive T cells that inhibit immune responses to self antigens(2). How these different types of T cell responses are induced after the interaction with DCs is an important issue to understand the pathogenesis of various immune-related disorders and to develop novel immunological therapies.

In humans, DCs are composed of two subsets: myeloid DCs (mDCs) and plasmacytoid DCs (pDCs). Recent studies have revealed that these DC subsets perform different functions in both innate and adaptive immune responses. At the same time, both of the DC subsets have functional plasticity to induce appropriate T cell responses depending on the types of stimuli. In this review, I discuss the diversity of DC subsets in humans from the perspective of their roles in (1) innate and adaptive immune responses and (2) tumor immunotherapy.

3. HUMAN DC SUBSETS

In human peripheral blood, two DC subsets have been recognized based on the expression of CD11c(3). The $CD11c^+$ cells in blood express myeloid markers such as CD13, CD33, and CD11b(3), whereas the $CD11c^-$ cells do not express significant levels of myeloid markers(3). Freshly isolated $CD11c^-$ cells have plasmacytoid morphology with well developed rough endoplasmic reticulum and Golgi apparatus(4). The $CD11c^+$ DCs are generally called myeloid DCs (mDCs) based on the expression of myeloid markers, whereas $CD11c^-$ DCs are called plasmacytoid DCs (pDCs) based on its plasmacytoid morphology at the DC precursor stage. mDCs are subdivided using several markers: BDCA-1 (CD1c), BDCA-3, and CD16(5, 6).

In addition to these primary DC-committed cells, it is well established that monocytes differentiate into immature DCs in the presence of granulocyte-macrophage colony stimulating-factor (GM-CSF) plus interleukin (IL)-4(7), or GM-CSF plus interferon (IFN)-alpha (8). It has been reported that mDCs in blood develop macrophage morphology as well as the expression of butyrate esterase and CD14 in response to macrophage-colony stimulating factor (M-CSF)(9), suggesting that at least a subpopulation of mDCs in blood is capable of differentiating into macrophages and is thus related to monocyte-derived DCs in terms of cellular origin. However, the precise relationship between mDCs in blood and monocyte-derived DCs remains to be determined. Here we designate $CD11c^+$ mDCs in peripheral blood as “blood mDCs”, and blood mDCs and monocyte-derived DCs collectively as “mDCs”.

4. DEVELOPMENTAL ORIGIN OF MDCS AND PDCS

The developmental origin of pDCs has been intensively studied. Initially, pDCs were considered to have lymphoid origin, based on the lack of myeloid markers(3, 4), the expression of mRNA for several lymphoid-related molecules (pT alpha, CD3, RAG1), and D-J rearrangement of a TCR beta gene locus(10). However, using an *in vitro* culture system, it has been shown that human pDCs can derive from both common myeloid progenitors (CMPs) and common lymphoid progenitors (CLPs)(11). Recently, using an *in vivo* system in which human CMPs and CLPs are transferred to immunodeficient mice, it has been shown that both CMPs and CLPs can give rise to pDCs as well as mDCs, and that transcriptional signatures of both DC subsets are preserved irrespective of their cell lineage origin(12). These studies suggest that DCs use unique developmental programs that cannot be categorized into the conventional myeloid or lymphoid pathway. It remains to be determined whether common mDC/pDC precursors exist.

5. FUNCTIONS OF MDCS AND PDCS IN INNATE IMMUNITY

Although type I IFN is the first cytokine reported and is essential in anti-viral immune responses(13), identification of the main producers of type I IFN in human blood had been elusive(14). We(15) and others(16) have found that pDC precursors produce vast amounts of type I IFNs (IFN-alpha/beta/omega), especially IFN-alpha (17), upon viral stimulation, thus representing the long-sought, main producers of type I IFNs in human blood. After producing type I IFNs upon viral stimulation, pDC precursors develop to DCs(17). During this process, pDC precursors produce most of the type I IFNs within 24 hours (18). Type I IFNs produced by pDC precursors are likely to play a crucial role in anti-viral immune responses(19).

Recent studies have revealed that the recognition of pathogen-associated molecules by cell types in the innate immune system through Toll-like receptors (TLRs) is the essential step towards the induction of innate immune responses followed by adaptive immune responses(20). Thus, we compared the expression patterns of TLR mRNA in different subsets of antigen-presenting cells (APCs), i.e. monocytes, blood mDCs, and pDC precursors(21). Importantly, pDC precursors exhibit distinct expression profiles of TLRs compared with monocytes and blood mDCs (21)(Figure 1). Strong expressions of mRNA were found in monocytes for TLR1, TLR2, TLR4, TLR5, and TLR8; in blood mDCs for TLR1, TLR2, TLR3, and TLR5; in pDC precursors for TLR7 and TLR9. Thus, myeloid APCs share several TLRs, whereas pDCs express distinct TLRs. Accordingly, monocytes respond to TLR2 ligands (peptidoglycan from gram-positive bacteria and lipoteichoic acid from various types of bacteria) and TLR4 ligand (lipopolysaccharide [LPS] from gram-negative bacteria) to produce TNF-alpha and IL-6(21). Blood mDCs respond to TLR3 ligand (poly I:C mimicking double-stranded RNA in virus-infected cells) to produce IFN-alpha/beta and IL-12p70(21, 22). pDC precursors respond to TLR7 ligand (anti-viral reagents imidazoquinolines(23) and viral single-stranded RNA as a physiological ligand(24, 25)) and TLR9 ligand (unmethylated CpG DNA from viruses and bacteria) to produce IFN-alpha(21, 22). Studies using murine pDCs have shown that TLR9 is responsible

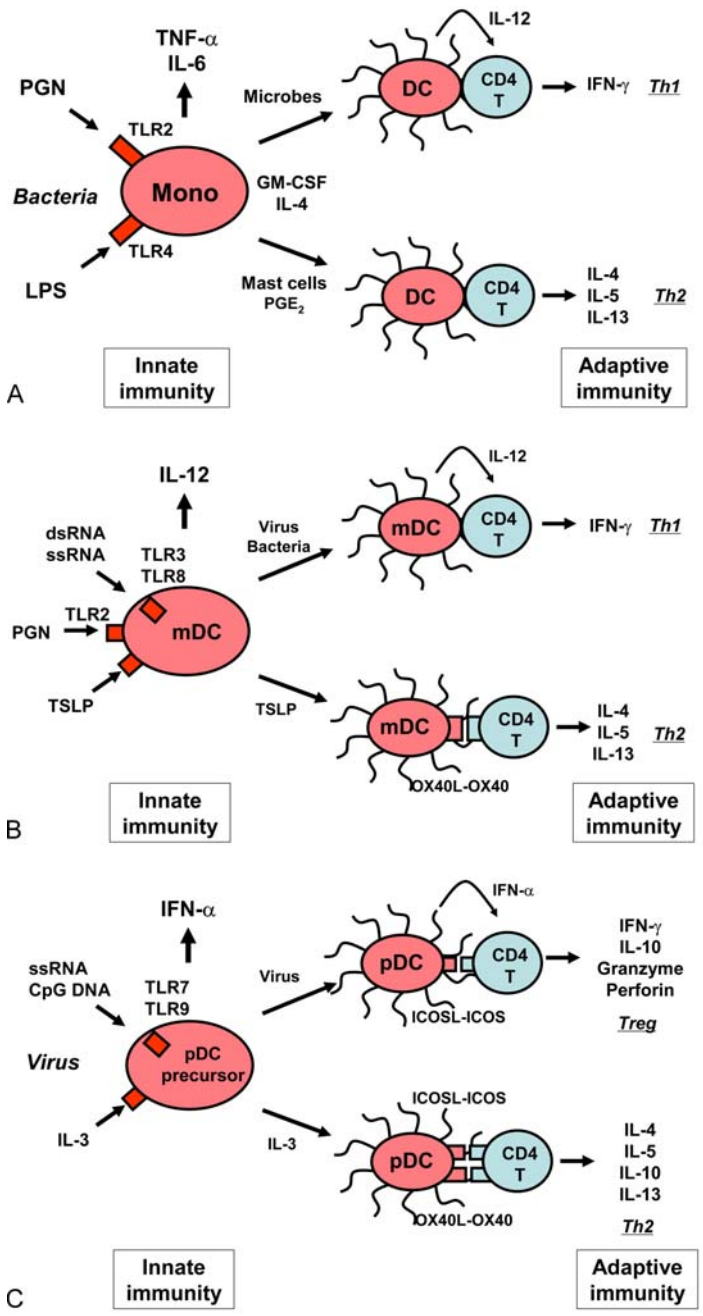

Figure 1. Human DC subsets induce different types of $CD4^+$ Th cell responses depending on environmental factors. (A) During innate immune responses, monocytes produce proinflammatory factors, such as TNF-alpha and IL-6, in response to bacterial components through TLRs. Thereafter, monocyte-derived DCs induced by GM-CSF and IL-4 produce IL-12 in response to certain microbes, and thus induce a Th1 response. In contrast, monocyte-derived DCs stimulated with mast cells or PGE_2 together with proinflammatory factors induce a Th2 response. (B) Myeloid DCs produce IL-12 in response to bacterial or viral components through TLRs and thus induce a Th1 response. In contrast, myeloid DCs stimulated with TSLP induce a Th2 response through the interaction between OX40 ligand and OX40. (C) During innate immune responses, plasmacytoid DC precursors produce a large amount of IFN-alpha in response to viral components through TLR7 and TLR9. Thereafter, plasmacytoid DCs stimulated with viruses induce IL-10-producing cytotoxic regulatory T cells, whereas plasmacytoid DCs stimulated with IL-3 induce a Th2 response through the interaction between OX40 ligand and OX40. Moreover, ICOS ligand on plasmacytoid DCs is responsible for the induction of IL-10 by T cells, which is important for an immunoregulatory function of the T cells. PGN: peptidoglycan, LPS: lipopolysaccharides, dsRNA: double-stranded RNA, ssRNA: single-stranded RNA.

for the recognition of viruses by pDC precursors(26, 27). Taken together, myeloid APCs (monocytes and blood mDCs) mainly recognize bacterial components and produce proinflammatory cytokines tumor necrosis factor (TNF)-alpha, IL-6, IL-12, whereas pDC precursors mainly recognize viral components and produce a large amount of type I IFNs (Figure 1). Thus, the two DC subsets may induce different modes of innate immunity suitable for eliminating either bacteria or viruses. In particular, because pDCs have poor endocytic activity(4, 28) but are able to present viral antigens(29, 30), these cells are well equipped to induce anti-viral innate and adaptive immune responses.

Due to the pleiotropic effects of type I IFNs on various immune cells(31), pDCs augment the functions of monocytes(32), blood mDCs(33), B cells(34), $CD8^+$ T cells(35), NK cells(36, 37), and NKT cells(38) *in vitro*. Monocytes differentiate into DCs in response to serum from lupus patients, and IFN-alpha in the serum is responsible for the differentiation into DCs, suggesting that pDCs are involved in the pathogenesis of lupus by producing IFN- alpha (32). Consistent with this, pDCs stimulated with CpG oligodeoxynucleotide (ODN) have been shown to induce monocytes to differentiate into DCs in an IFN-alpha-dependent manner(39, 40). HIV-activated pDCs induce maturation of blood mDCs by producing IFN-alpha and TNF-alpha(33). pDCs induce B cells to differentiate into immunoglobulin-secreting plasma cells by producing IFN-alpha and IL-6(34). pDCs stimulated with CpG ODN enhance proliferation of peptide-specific $CD8^+$ T cells(35), and promote NK cell cytotoxicity and IFN-gamma production through type I IFN and the ligand for glucocorticoid-induced tumor necrosis factor receptor (GITRL) (36, 37). When NKT cells were preincubated with a pDC-conditioned supernatant, there was an increase in the proliferation of NKT cells cocultured with alpha-galactosylceramide-loaded mDCs(38). These studies indicate that pDCs play a pivotal role in augmenting cellular and humoral immune responses by producing the pleiotropic cytokine, IFN-alpha.

6. INDUCTION OF DIFFERENT TYPES OF ADAPTIVE T CELL RESPONSES BY MDCS

The human immune system has evolved to have two separate mechanisms for protection against different types of microbes. In response to intracellular microbes, such as bacteria, viruses, and intracellular parasites, DCs are induced to produce IL-12 and type I IFNs (41, 42). These activated DCs can then stimulate $CD4^+$ Th cells to differentiate into IFN-gamma-producing Th1 cells (43, 44). The activated Th1 cells, in turn, help to activate macrophages and $CD8^+$ cytotoxic T cells to kill intracellular microbes. In response to extracellular parasites, such as helminthes, activated DCs induce $CD4^+$ Th cells to differentiate into Th2 cells (41, 45, 46). Th2 cells produce proallergic cytokines such as IL-4, IL-5, and IL-13, which trigger IgE production. IgE, in turn, activates mast cells and eosinophils to eradicate the extracellular microbes.

DCs play a critical role in directing different effector T cell responses (43, 47). mDCs have a capacity to produce IL-12 in response to the microbial stimuli and, thereby, to induce Th1 development(48). However, this capacity varies with the type of signals delivered to DCs (Figure 1A, B). For example, LPS derived from *Escherichia coli* (49, 50), *Mycobacterium tuberculosis* (51), and double-stranded viral RNA (21, 52, 53), all activate mDCs to produce IL-12 and to induce Th1 development, whereas LPS from *Porphyromonas gingivalis* (49, 54), Der p 1 (house dust mite allergen) (55, 56), and *Schistosoma mansoni* egg extract (52, 57, 58), all activate mDCs to induce Th2 development, which is associated with a lower capacity of the DCs to produce IL-12. Thus, different microbes and their components induce mDCs to produce different levels of IL-12, which leads to different types of Th responses appropriate to eliminate given pathogens.

Environmentally bioactive substances produced by inflammatory processes that act as a cyclic AMP upregulator, such as prostaglandin (PG) E_2(58) and histamine(59), enhance TNF-alpha-dependent mDC maturation but suppresses bioactive IL-12 p70 production, resulting in Th2 responses (Figure 1A).

Due to the proximity of DCs and mast cells in skin and mucosa, activation of mast cells may affect DC functions. Indeed, we showed that IgE-activated mast cells in combination with proinflammatory factors induce IL-12-nonproducing, Th2-promoting mDCs (60) (Figure 1A). Thus, the interaction between DCs and IgE-activated mast cells in a proinflammatory environment may be instrumental in maintaining and augmenting Th2 responses in allergy.

Thymic stromal lymphopoietin (TSLP), an IL-7-like cytokine, may be a key physiological mediator that cause allergic inflammation through DCs. TSLP strongly activates human blood mDCs to upregulate costimulatory molecules and to secrete Th2-attracting chemokines TARC and MDC, but neither IL-12 nor pro-inflammatory cytokines (61, 62). These TSLP-activated DCs in turn induce allogeneic naïve $CD4^+$ T cells to undergo robust proliferation and to differentiate into Th2 cells by expressing OX40 ligand(63) (Figure 1B). These findings, together with high TSLP expression in keratinocytes from the skin lesions of patients with atopic dermatitis (62), suggest that TSLP plays a critical role in the initiation of allergic inflammation.

Immunosuppressive cytokines such as IL-10 and transforming growth factor (TGF)-beta, as well as steroid, cyclosporin A, and 1-alpha, 25-dihydoxyvitamin D3, inhibit the maturation of mDCs as well as their IL-12 production (64-69). These DCs cannot drive DC-mediated Th1 responses but rather induce DC-mediated regulatory T cells (or possibly Th2 cells), similar to the immature steady-state DCs (70, 71).

Taken together, mDCs are capable of inducing naïve $CD4^+$ T cells to differentiate into Th1, Th2, and even regulatory T cells, depending on the stimuli the DCs

receive from the environment. IL-12 is a dominant factor in inducing a Th1 response.

7. INDUCTION OF DIFFERENT TYPES OF ADAPTIVE T CELL RESPONSES BY PDCS

Following an innate immune response, pDCs switch their functional properties from type I IFN producers to mature DCs that directly modulate T cell functions. Signaling through TLR7 and TLR9 by viruses or by synthetic ligands can stimulate pDCs to produce IFN-alpha/beta and rapidly undergo maturation (17, 22, 72).

Virus-stimulated pDCs induce human naïve $CD4^+$ allogeneic T cells to differentiate into IFN-gamma- and IL-10-producing cells (17), which have an immunoregulatory function(73) (Figure 1C). In contrast to pDCs stimulated with virus or CpG ODN, IL-3-stimulated pDCs do not produce IFN-alpha, and prime naïve $CD4^+$ T cells to produce Th2 cytokines by expressing OX40 ligand (4, 17, 74) (Figure 1C). The biological significance of pDC differentiation into mature DCs in the presence of IL-3 is unclear.

Several studies have indicated tolerogenic functions of pDCs. The pDCs induced by IL-3 with CD40 ligand prime naive $CD8^+$ T cells to differentiate into IL-10-producing suppressor T cells, which inhibit bystander proliferation of $CD8^+$ T cells in an IL-10-dependent manner (75). Recent studies have shown that, in humans, freshly isolated pDC precursors induce $CD4^+$ T cell anergy (76) and pDC-derived DCs activated by CpG ODN prime naïve $CD4^+$ T cells to differentiate into $CD4^+CD25^+$ regulatory T cells characterized as $Foxp3^+$ IL-10-producing suppressor T cells (77). We have also shown that virus-stimulated pDCs induce naïve $CD4^+$ T cells to differentiate into IFN-gamma- and IL-10-producing cytotoxic regulatory T cells that express granzymes and perforin(73) (Figure 1C). Thus, pDCs that have been stimulated with various stimuli induce IL-10-producing regulatory T cells. Of note, it has been shown that inducible costimulator (ICOS) ligand highly expressed on pDCs is responsible for the IL-10 production by T cells(78) (Figure 1C). This finding indicates that pDCs have an intrinsic ability to induce IL-10-producing regulatory T cells by expressing ICOS ligand, which discriminates pDCs from mDCs.

In anti-viral immunity, pDCs appear to be capable of priming influenza virus-specific primary and secondary $CD4^+$ and $CD8^+$ T cell immune responses *in vitro* and *in vivo* (29, 30). Although pDCs have been considered to be have a poor capacity to cross-present exogenous antigens on MHC class I molecules(79) partly due to their poor phagocytic activity (4, 28), it has recently been shown that pDCs can cross-present HIV antigens from apoptotic cells as efficiently as mDCs(80). Given the tolerogenic activity of pDCs repeatedly shown by the *in vitro* studies, it remains to be determined whether such presentation of viral antigens leads to T cell stimulation or T cell anergy *in vivo*.

8. APPLICATION OF HUMAN DC SUBSETS TO IMMUNOTHERAPY

A variety of ligands for TLRs is under clinical development as adjuvants in immunotherapy for cancer, infection, and allergy(81). Due to the broad range of TLR expression, DCs represent principal targets of such adjuvants. In particular, TLR9 ligands, CpG ODNs, which induce pDCs to produce type I IFNs, are promising adjuvants due to multiple immunostimulatory effects of type I IFNs. In addition, topical administration of imidazoquinolines, which stimulate pDCs and mDCs through TLR7 and TLR8, respectively, and induce recruitment of pDCs to the skin(82), is under clinical trials for skin cancers (basal cell carcinoma and melanoma)(81). Infiltration of mDCs and pDCs has been reported in tumors lesions, such as ovarian cancer(83), melanoma(84), head and neck cancer(85), and lung cancer(86). The pDCs in tumor sites under steady state are incapable of activating T cells and instead induce IL-10-producing regulatory T cells(83, 87). It is possible that stimulation of pDCs in tumor sites with CpG ODNs activates adjacent mDCs through type I IFNs, which may evoke tumor-specific immune responses.

There are three classes of immunostimulatory CpG ODNs(88): A class ODN that potently stimulates pDCs to produce IFN-alpha, B class ODN that stimulates B cells and pDCs with a much lower level of IFN-alpha production than A class, and C class ODN that stimulates both B cells and pDCs with a moderate level of IFN-alpha production. B class ODNs have been used in clinical trials for B-cell lymphoma (in combination with anti-CD20 monoclonal antibody, rituximab)(89), melanoma (in combination with MART-1 peptide)(90), and non-small cell lung cancer (in combination with chemotherapy)(88). The administration of CpG ODNs was safe, and remarkable increases in the number of MART-1-specific $CD8^+$ T cells were induced in the melanoma patients(90), indicating the potency of pDC activation in enhancing antigen-specific T cell responses.

CpG ODNs and imidazoquinolines are under clinical trials for viral infections, such as hepatitis B, hepatitis C, HIV, genital herpes, and influenza(81). CpG ODNs are also used for allergic rhinitis and asthma in an attempt to counterbalance Th2 responses with vaccine-induced Th1 responses(81).

Administration of myeloid DCs, mainly monocyte-derived DCs, which were pulsed with tumor antigens in the form of peptide, mRNA encoding tumor antigens, tumor lysate, or apoptotic tumor cells, has been widely applied for various types of cancers, and exhibits clinical responses in about 10% of patients(91).

9. SUMMARY AND PERSPECTIVE

In host defense, many of the “danger signals” of infection are integrated by DCs and then converted into appropriate types of immune responses. The specialized roles of each DC subset in innate and adaptive immune

responses have been well characterized in this decade. In the future, further knowledge of the molecular mechanisms that control functional plasticity of the DC system may lead to improvements in the treatment of a wide variety of diseases, such as cancers, infections, allergy, and autoimmune disorders.

So far, for clinical trials, DCs have been mainly used for tumor immunotherapy. DCs have been prepared *ex vivo* in many cases, but it takes substantial cost and labor. Immunotherapy using DCs prepared *ex vivo* is still need to be pursued in an attempt to improve clinical outcomes, particularly by applying DC therapy to patients with minimal tumor burden. For the future, antigens will be targeted to DCs *in vivo* by administrating a tumor antigen conjugated with a DC-targeting component, such as an antibody specific to surface molecules on DCs (92). In this case, induction of DC maturation using appropriate adjuvants is mandatory to induce anti-tumor immunity instead of tolerance. In this context, activation of both mDCs and pDCs in appropriate sites, i.e. tumor sites and lymphoid tissues, using appropriate TLR ligands will synergistically augment anti-tumor immune responses. Development of technology that realizes such advanced immunotherapy is expected in the near future.

10. REFERENCES

1. Banchereau, F. Briere, C. Caux, J. Davoust, S. Lebecque, Y. J. Liu, B. Pulendran and K. Palucka: Immunobiology of dendritic cells. *Annu Rev Immunol* 18, 767-811 (2000)

2. R. M. Steinman, D. Hawiger and M. C. Nussenzweig: Tolerogenic dendritic cells. *Annu Rev Immunol* 21, 685-711 (2003)

3. U. O'Doherty, M. Peng, S. Gezelter, W. J. Swiggard, M. Betjes, N. Bhardwaj and R. M. Steinman: Human blood contains two subsets of dendritic cells, one immunologically mature and the other immature. *Immunology* 82, 487-493 (1994)

4. G. Grouard, M. C. Rissoan, L. Filgueira, I. Durand, J. Banchereau and Y. J. Liu: The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin (IL)-3 and CD40-ligand. *J Exp Med* 185, 1101-1111 (1997)

5. K. P. A. MacDonald, D. J. Munster, G. J. Clark, A. Dzionek, J. Schmitz and D. N. J. Hart: Characterization of human blood dendritic cell subsets. *Blood* 100, 4512-4520 (2002)

6. D. Piccioli, S. Tavarini, E. Borgogni, V. Steri, S. Nuti, C. Sammicheli, M. Bardelli, D. Montagna, F. Locatelli and A. Wack: Functional specialization of human circulating CD16 and CD1c myeloid dendritic-cell subsets. *Blood* 109, 5371-5379 (2007)

7. F. Sallusto and A. Lanzavecchia: Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J Exp Med* 179, 1109-1118 (1994)

8. S. M. Santini, C. Lapenta, M. Logozzi, S. Parlato, M. Spada, T. Di Pucchio and F. Belardelli: Type I interferon as a powerful adjuvant for monocyte-derived dendritic cell development and activity *in vitro* and in Hu-PBL-SCID mice. *J Exp Med* 191, 1777-1788 (2000)

9. S. P. Robinson, S. Patterson, N. English, D. Davies, S. C. Knight and C. D. Reid: Human peripheral blood contains two distinct lineages of dendritic cells. *Eur J Immunol* 29, 2769-2778 (1999)

10. L. Bruno, P. Res, M. Dessing, M. Cella and H. Spits: Identification of a committed T cell precursor population in adult human peripheral blood. *J Exp Med* 185, 875-884 (1997)

11. L. Chicha, D. Jarrossay and M. G. Manz: Clonal type I interferon-producing and dendritic cell precursors are contained in both human lymphoid and myeloid progenitor populations. *J Exp Med* 200, 1519-1524 (2004)

12. F. Ishikawa, H. Niiro, T. Iino, S. Yoshida, N. Saito, S. Onohara, T. Miyamoto, H. Minagawa, S.-i. Fujii, L. D. Shultz, M. Harada and K. Akashi: The developmental program of human dendritic cells is operated independently of conventional myeloid and lymphoid pathways. *Blood* 110, 3591-3660 (2007)

13. U. Muller, U. Steinhoff, L. F. Reis, S. Hemmi, J. Pavlovic, R. M. Zinkernagel and M. Aguet: Functional role of type I and type II interferons in antiviral defense. *Science* 264, 1918-1921 (1994)

14. P. Fitzgerald-Bocarsly: Human natural interferon-alpha producing cells. *Pharmacol Ther* 60, 39-62 (1993)

15. F. P. Siegal, N. Kadowaki, M. Shodell, P. A. Fitzgerald-Bocarsly, K. Shah, S. Ho, S. Antonenko and Y. J. Liu: The nature of the principal type 1 interferon-producing cells in human blood. *Science* 284, 1835-1837 (1999)

16. M. Cella, D. Jarrossay, F. Facchetti, O. Alebardi, H. Nakajima, A. Lanzavecchia and M. Colonna: Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nat Med* 5, 919-923 (1999)

17. N. Kadowaki, S. Antonenko, J. Y. Lau and Y. J. Liu: Natural interferon alpha/beta-producing cells link innate and adaptive immunity. *J Exp Med* 192, 219-226 (2000)

18. D. Jarrossay, G. Napolitani, M. Colonna, F. Sallusto and A. Lanzavecchia: Specialization and complementarity in microbial molecule recognition by human myeloid and plasmacytoid dendritic cells. *Eur J Immunol* 31, 3388-3393 (2001)

19. M. Dalod, T. P. Salazar-Mather, L. Malmgaard, C. Lewis, C. Asselin-Paturel, F. Briere, G. Trinchieri and C. A. Biron: Interferon alpha/beta and interleukin 12 responses to viral infections: pathways regulating dendritic cell cytokine expression *in vivo*. *J Exp Med* 195, 517-528 (2002)

20. A. Iwasaki and R. Medzhitov: Toll-like receptor control of the adaptive immune responses. *Nat Immunol* 5, 987-995 (2004)

21. N. Kadowaki, S. Ho, S. Antonenko, R. de Waal Malefyt, R. A. Kastelein, F. Bazan and Y.-J. Liu: Subsets of Human Dendritic Cell Precursors Express Different Toll-like Receptors and Respond to Different Microbial Antigens. *J Exp Med* 194, 863-870 (2001)

22. N. Kadowaki, S. Antonenko and Y. J. Liu: Distinct CpG DNA and Polyinosinic-Polycytidylic Acid Double-Stranded RNA, Respectively, Stimulate CD11c- Type 2 Dendritic Cell Precursors and CD11c+ Dendritic Cells to Produce Type I IFN. *J Immunol* 166, 2291-2295 (2001)

23. T. Ito, R. Amakawa, T. Kaisho, H. Hemmi, K. Tajima, K. Uehira, Y. Ozaki, H. Tomizawa, S. Akira and S. Fukuhara: Interferon-alpha and interleukin-12 are induced differentially by Toll-like receptor 7 ligands in human blood dendritic cell subsets. *J Exp Med* 195, 1507-1512 (2002)

24. F. Heil, H. Hemmi, H. Hochrein, F. Ampenberger, C. Kirschning, S. Akira, G. Lipford, H. Wagner and S. Bauer: Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. *Science* 303, 1526-1529 (2004)

25. S. S. Diebold, T. Kaisho, H. Hemmi, S. Akira and C. Reis e Sousa: Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science* 303, 1529-1531 (2004)

26. J. Lund, A. Sato, S. Akira, R. Medzhitov and A. Iwasaki: Toll-like receptor 9-mediated recognition of Herpes simplex virus-2 by plasmacytoid dendritic cells. *J Exp Med* 198, 513-520 (2003)

27. A. Krug, G. D. Luker, W. Barchet, D. A. Leib, S. Akira and M. Colonna: Herpes simplex virus type 1 activates murine natural interferon-producing cells through toll-like receptor 9. *Blood* 103, 1433-1437 (2004)

28. T. Ito, M. Inaba, K. Inaba, J. Toki, S. Sogo, T. Iguchi, Y. Adachi, K. Yamaguchi, R. Amakawa, J. Valladeau, S. Saeland, S. Fukuhara and S. Ikehara: A CD1a+/CD11c+ subset of human blood dendritic cells is a direct precursor of Langerhans cells. *J Immunol* 163, 1409-1419 (1999)

29. J.-F. Fonteneau, M. Gilliet, M. Larsson, I. Dasilva, C. Munz, Y.-J. Liu and N. Bhardwaj: Activation of influenza virus-specific CD4+ and CD8+ T cells: a new role for plasmacytoid dendritic cells in adaptive immunity. *Blood* 101, 3520-3526 (2003)

30. G. Schlecht, S. Garcia, N. Escriou, A. A. Freitas, C. Leclerc and G. Dadaglio: Murine plasmacytoid dendritic cells induce effector/memory CD8+ T-cell responses *in vivo* after viral stimulation. *Blood* 104, 1808-1815 (2004)

31. L. M. Pfeffer, C. A. Dinarello, R. B. Herberman, B. R. Williams, E. C. Borden, R. Bordens, M. R. Walter, T. L. Nagabhushan, P. P. Trotta and S. Pestka: Biological properties of recombinant alpha-interferons: 40th anniversary of the discovery of interferons. *Cancer Res* 58, 2489-2499 (1998)

32. P. Blanco, A. K. Palucka, M. Gill, V. Pascual and J. Banchereau: Induction of dendritic cell differentiation by IFN-alpha in systemic lupus erythematosus. *Science* 294, 1540-1543 (2001)

33. J.-F. Fonteneau, M. Larsson, A.-S. Beignon, K. McKenna, I. Dasilva, A. Amara, Y.-J. Liu, J. D. Lifson, D. R. Littman and N. Bhardwaj: Human Immunodeficiency Virus Type 1 Activates Plasmacytoid Dendritic Cells and Concomitantly Induces the Bystander Maturation of Myeloid Dendritic Cells. *J. Virol.* 78, 5223-5232 (2004)

34. G. Jego, A. K. Palucka, J. P. Blanck, C. Chalouni, V. Pascual and J. Banchereau: Plasmacytoid dendritic cells induce plasma cell differentiation through type I interferon and interleukin 6. *Immunity* 19, 225-234 (2003)

35. S. Rothenfusser, V. Hornung, M. Ayyoub, S. Britsch, A. Towarowski, A. Krug, A. Sarris, N. Lubenow, D. Speiser, S. Endres and G. Hartmann: CpG-A and CpG-B oligonucleotides differentially enhance human peptide-specific primary and memory CD8+ T-cell responses *in vitro*. *Blood* 103, 2162-2169 (2004)

36. F. Gerosa, A. Gobbi, P. Zorzi, S. Burg, F. Briere, G. Carra and G. Trinchieri: The Reciprocal Interaction of NK Cells with Plasmacytoid or Myeloid Dendritic Cells Profoundly Affects Innate Resistance Functions. *J Immunol* 174, 727-734 (2005)

37. S. Hanabuchi, N. Watanabe, Y.-H. Wang, Y.-H. Wang, T. Ito, J. Shaw, W. Cao, F. X.-F. Qin and Y.-J. Liu: Human plasmacytoid predendritic cells activate NK cells through glucocorticoid-induced tumor necrosis factor receptor-ligand (GITRL). *Blood* 107, 3617-3623 (2006)

38. C. J. Montoya, H.-B. Jie, L. Al-Harthi, C. Mulder, P. J. Patino, M. T. Rugeles, A. M. Krieg, A. L. Landay and S. B. Wilson: Activation of Plasmacytoid Dendritic Cells with TLR9 Agonists Initiates Invariant NKT Cell-Mediated Cross-Talk with Myeloid Dendritic Cells. *J Immunol* 177, 1028-1039 (2006)

39. D. V. D. M. K. Mayda Gursel: CpG oligodeoxynucleotides induce human monocytes to mature into functional dendritic cells. *European Journal of Immunology* 32, 2617-2622 (2002)

40. A. Krug, S. Rothenfusser, S. Selinger, C. Bock, M. Kerkmann, J. Battiany, A. Sarris, T. Giese, D. Speiser, S.

Endres and G. Hartmann: CpG-A Oligonucleotides Induce a Monocyte-Derived Dendritic Cell-Like Phenotype That Preferentially Activates CD8 T Cells. *J Immunol* 170, 3468-3477 (2003)

41. J. Banchereau and R. M. Steinman: Dendritic cells and the control of immunity. *Nature* 392, 245-252 (1998)

42. B. Pulendran, K. Palucka and J. Banchereau: Sensing pathogens and tuning immune responses. *Science* 293, 253-256 (2001)

43. A. Lanzavecchia and F. Sallusto: Regulation of T cell immunity by dendritic cells. *Cell* 106, 263-266 (2001)

44. S. C. Eisenbarth, D. A. Piggott and K. Bottomly: The master regulators of allergic inflammation: dendritic cells in Th2 sensitization. *Curr Opin Immunol* 15, 620-626 (2003)

45. M. Moser and K. M. Murphy: Dendritic cell regulation of TH1-TH2 development. *Nat Immunol* 1, 199-205. (2000)

46. A. Sher, E. Pearce and P. Kaye: Shaping the immune response to parasites: role of dendritic cells. *Curr Opin Immunol* 15, 421-429 (2003)

47. Y. J. Liu, H. Kanzler, V. Soumelis and M. Gilliet: Dendritic cell lineage, plasticity and cross-regulation. *Nat Immunol* 2, 585-589 (2001)

48. T. Ito, H. Kanzler, O. Duramad, W. Cao and Y.-J. Liu: Specialization, kinetics, and repertoire of type 1 interferon responses by human plasmacytoid predendritic cells. *Blood* 107, 2423-2431 (2006)

49. B. Pulendran, P. Kumar, C. W. Cutler, M. Mohamadzadeh, T. Van Dyke and J. Banchereau: Lipopolysaccharides from distinct pathogens induce different classes of immune responses *in vivo*. *J Immunol* 167, 5067-5076 (2001)

50. H. Qi, T. L. Denning and L. Soong: Differential induction of interleukin-10 and interleukin-12 in dendritic cells by microbial toll-like receptor activators and skewing of T-cell cytokine profiles. *Infect Immun* 71, 3337-3342 (2003)

51. S. Stenger and R. L. Modlin: Control of Mycobacterium tuberculosis through mammalian Toll-like receptors. *Curr Opin Immunol* 14, 452-457 (2002)

52. E. C. de Jong, P. L. Vieira, P. Kalinski, J. H. Schuitemaker, Y. Tanaka, E. A. Wierenga, M. Yazdanbakhsh and M. L. Kapsenberg: Microbial compounds selectively induce Th1 cell-promoting or Th2 cell-promoting dendritic cells *in vitro* with diverse th cell-polarizing signals. *J Immunol* 168, 1704-1709 (2002)

53. M. Cella, M. Salio, Y. Sakakibara, H. Langen, I. Julkunen and A. Lanzavecchia: Maturation, activation, and protection of dendritic cells induced by double-stranded RNA. *J Exp Med* 189, 821-829 (1999)

54. R. Jotwani, B. Pulendran, S. Agrawal and C. W. Cutler: Human dendritic cells respond to Porphyromonas gingivalis LPS by promoting a Th2 effector response *in vitro*. *Eur J Immunol* 33, 2980-2986 (2003)

55. H. Hammad, H. H. Smits, C. Ratajczak, A. Nithiananthan, E. A. Wierenga, G. A. Stewart, A. Jacquet, A. B. Tonnel and J. Pestel: Monocyte-derived dendritic cells exposed to Der p 1 allergen enhance the recruitment of Th2 cells: major involvement of the chemokines TARC/CCL17 and MDC/CCL22. *Eur Cytokine Netw* 14, 219-228 (2003)

56. H. Hammad, A. S. Charbonnier, C. Duez, A. Jacquet, G. A. Stewart, A. B. Tonnel and J. Pestel: Th2 polarization by Der p 1--pulsed monocyte-derived dendritic cells is due to the allergic status of the donors. *Blood* 98, 1135-1141 (2001)

57. C. M. Kane, L. Cervi, J. Sun, A. S. McKee, K. S. Masek, S. Shapira, C. A. Hunter and E. J. Pearce: Helminth antigens modulate TLR-initiated dendritic cell activation. *J Immunol* 173, 7454-7461 (2004)

58. P. Kalinski, P. L. Vieira, J. H. Schuitemaker, E. C. de Jong and M. L. Kapsenberg: Prostaglandin E (2) is a selective inducer of interleukin-12 p40 (IL-12p40) production and an inhibitor of bioactive IL-12p70 heterodimer. *Blood* 97, 3466-3469 (2001)

59. M. Idzko, A. la Sala, D. Ferrari, E. Panther, Y. Herouy, S. Dichmann, M. Mockenhaupt, F. Di Virgilio, G. Girolomoni and J. Norgauer: Expression and function of histamine receptors in human monocyte-derived dendritic cells. *J Allergy Clin Immunol* 109, 839-846 (2002)

60. T. Kitawaki, N. Kadowaki, N. Sugimoto, N. Kambe, T. Hori, Y. Miyachi, T. Nakahata and T. Uchiyama: IgE-activated mast cells in combination with pro-inflammatory factors induce Th2-promoting dendritic cells. *Int. Immunol.* 18, 1789-1799 (2006)

61. P. A. Reche, V. Soumelis, D. M. Gorman, T. Clifford, M. Liu, M. Travis, S. M. Zurawski, J. Johnston, Y. J. Liu, H. Spits, R. de Waal Malefyt, R. A. Kastelein and J. F. Bazan: Human thymic stromal lymphopoietin preferentially stimulates myeloid cells. *J Immunol* 167, 336-343 (2001)

62. V. Soumelis, P. A. Reche, H. Kanzler, W. Yuan, G. Edward, B. Homey, M. Gilliet, S. Ho, S. Antonenko, A. Lauerma, K. Smith, D. Gorman, S. Zurawski, J. Abrams, S. Menon, T. McClanahan, R. de Waal-Malefyt Rd, F. Bazan, R. A. Kastelein and Y. J. Liu: Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 3, 673-680 (2002)

63. T. Ito, Y. H. Wang, O. Duramad, T. Hori, G. J. Delespesse, N. Watanabe, F. X. Qin, Z. Yao, W. Cao and Y. J. Liu: TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J Exp Med* 202, 1213-1223 (2005)

64. T. De Smedt, M. Van Mechelen, G. De Becker, J. Urbain, O. Leo and M. Moser: Effect of interleukin-10 on dendritic cell maturation and function. *Eur J Immunol* 27, 1229-1235 (1997)

65. K. Steinbrink, M. Wolfl, H. Jonuleit, J. Knop and A. H. Enk: Induction of tolerance by IL-10-treated dendritic cells. *J Immunol* 159, 4772-4780 (1997)

66. H. Strobl and W. Knapp: TGF-beta1 regulation of dendritic cells. *Microbes Infect* 1, 1283-1290 (1999)

67. D. Sauma, A. Fierro, J. R. Mora, A. M. Lennon-Dumenil, M. R. Bono, M. Rosemblatt and J. Morales: Cyclosporine preconditions dendritic cells during differentiation and reduces IL-2 and IL-12 production following activation: a potential tolerogenic effect. *Transplant Proc* 35, 2515-2517 (2003)

68. H. Matsue, C. Yang, K. Matsue, D. Edelbaum, M. Mummert and A. Takashima: Contrasting impacts of immunosuppressive agents (rapamycin, FK506, cyclosporin A, and dexamethasone) on bidirectional dendritic cell-T cell interaction during antigen presentation. *J Immunol* 169, 3555-3564 (2002)

69. A. E. Pedersen, M. Gad, M. R. Walter and M. H. Claesson: Induction of regulatory dendritic cells by dexamethasone and 1alpha,25-Dihydroxyvitamin D (3). *Immunol Lett* 91, 63-69 (2004)

70. H. Jonuleit, E. Schmitt, G. Schuler, J. Knop and A. H. Enk: Induction of interleukin 10-producing, nonproliferating CD4 (+) T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. *J Exp Med* 192, 1213-1222 (2000)

71. M. V. Dhodapkar, R. M. Steinman, J. Krasovsky, C. Munz and N. Bhardwaj: Antigen-specific inhibition of effector T cell function in humans after injection of immature dendritic cells. *J Exp Med* 193, 233-238 (2001)

72. A. Krug, A. Towarowski, S. Britsch, S. Rothenfusser, V. Hornung, R. Bals, T. Giese, H. Engelmann, S. Endres, A. M. Krieg and G. Hartmann: Toll-like receptor expression reveals CpG DNA as a unique microbial stimulus for plasmacytoid dendritic cells which synergizes with CD40 ligand to induce high amounts of IL-12. *Eur J Immunol* 31, 3026-3037 (2001)

73. K. Kawamura, N. Kadowaki, T. Kitawaki and T. Uchiyama: Virus-stimulated plasmacytoid dendritic cells induce CD4+ cytotoxic regulatory T cells. *Blood* 107, 1031-1038 (2006)

74. T. Ito, R. Amakawa, M. Inaba, T. Hori, M. Ota, K. Nakamura, M. Takebayashi, M. Miyaji, T. Yoshimura, K. Inaba and S. Fukuhara: Plasmacytoid dendritic cells regulate Th cell responses through OX40 ligand and type I IFNs. *J Immunol* 172, 4253-4259 (2004)

75. M. Gilliet and Y.-J. Liu: Generation of Human CD8 T Regulatory Cells by CD40 Ligand-activated Plasmacytoid Dendritic Cells. *J. Exp. Med.* 195, 695-704 (2002)

76. M. Kuwana, J. Kaburaki, T. M. Wright, Y. Kawakami and Y. Ikeda: Induction of antigen-specific human CD4 (+) T cell anergy by peripheral blood DC2 precursors. *Eur J Immunol* 31, 2547-2557 (2001)

77. E. A. Moseman, X. Liang, A. J. Dawson, A. Panoskaltsis-Mortari, A. M. Krieg, Y.-J. Liu, B. R. Blazar and W. Chen: Human Plasmacytoid Dendritic Cells Activated by CpG Oligodeoxynucleotides Induce the Generation of CD4+CD25+ Regulatory T Cells. *J Immunol* 173, 4433-4442 (2004)

78. T. Ito, M. Yang, Y.-H. Wang, R. Lande, J. Gregorio, O. A. Perng, X.-F. Qin, Y.-J. Liu and M. Gilliet: Plasmacytoid dendritic cells prime IL-10-producing T regulatory cells by inducible costimulator ligand. *J. Exp. Med.* 204, 105-115 (2007)

79. M. Schnurr, Q. Chen, A. Shin, W. Chen, T. Toy, C. Jenderek, S. Green, L. Miloradovic, D. Drane, I. D. Davis, J. Villadangos, K. Shortman, E. Maraskovsky and J. Cebon: Tumor antigen processing and presentation depend critically on dendritic cell type and the mode of antigen delivery. *Blood* 105, 2465-2472 (2005)

80. G. Hoeffel, A.-C. Ripoche, D. Matheoud, M. Nascimbeni, N. Escriou, P. Lebon, F. Heshmati, J.-G. Guillet, M. Gannage, S. Caillat-Zucman, N. Casartelli, O. Schwartz, H. De la Salle, D. Hanau, A. Hosmalin and C. Maranon: Antigen Crosspresentation by Human Plasmacytoid Dendritic Cells. *Immunity* 27, 481 (2007)

81. H. Kanzler, F. J. Barrat, E. M. Hessel and R. L. Coffman: Therapeutic targeting of innate immunity with Toll-like receptor agonists and antagonists. *Nat Med* 13, 552 (2007)

82. M. Urosevic, R. Dummer, C. Conrad, M. Beyeler, E. Laine, G. Burg and M. Gilliet: Disease-Independent Skin Recruitment and Activation of Plasmacytoid Predendritic Cells Following Imiquimod Treatment. *J. Natl. Cancer Inst.* 97, 1143-1153 (2005)

83. W. Zou, V. Machelon, A. Coulomb-L'Hermin, J. Borvak, F. Nome, T. Isaeva, S. Wei, R. Krzysiek, I. Durand-Gasselin, A. Gordon, T. Pustilnik, D. T. Curiel, P. Galanaud, F. Capron, D. Emilie and T. J. Curiel: Stromal-derived factor-1 in human tumors recruits and alters the function of plasmacytoid precursor dendritic cells. *Nat Med* 7, 1339-1346 (2001)

84. W. Vermi, R. Bonecchi, F. Facchetti, D. Bianchi, S. Sozzani, S. Festa, A. Berenzi, M. Cella and M. Colonna: Recruitment of immature plasmacytoid dendritic cells (plasmacytoid monocytes) and myeloid dendritic cells in primary cutaneous melanomas. *J Pathol* 200, 255-268 (2003)

85. E. Hartmann, B. Wollenberg, S. Rothenfusser, M. Wagner, D. Wellisch, B. Mack, T. Giese, O. Gires, S. Endres and G. Hartmann: Identification and Functional Analysis of Tumor-Infiltrating Plasmacytoid Dendritic Cells in Head and Neck Cancer. *Cancer Res* 63, 6478-6487 (2003)

86. I. Perrot, D. Blanchard, N. Freymond, S. Isaac, B. Guibert, Y. Pacheco and S. Lebecque: Dendritic Cells Infiltrating Human Non-Small Cell Lung Cancer Are Blocked at Immature Stage. *J Immunol* 178, 2763-2769 (2007)

87. S. Wei, I. Kryczek, L. Zou, B. Daniel, P. Cheng, P. Mottram, T. Curiel, A. Lange and W. Zou: Plasmacytoid Dendritic Cells Induce CD8+ Regulatory T Cells In Human Ovarian Carcinoma. *Cancer Res* 65, 5020-5026 (2005)

88. A. M. Krieg: Therapeutic potential of Toll-like receptor 9 activation. *Nat Rev Drug Discov* 5, 471 (2006)

89. J. W. Friedberg, H. Kim, M. McCauley, E. M. Hessel, P. Sims, D. C. Fisher, L. M. Nadler, R. L. Coffman and A. S. Freedman: Combination immunotherapy with a CpG oligonucleotide (1018 ISS) and rituximab in patients with non-Hodgkin lymphoma: increased interferon-{alpha}/{beta}-inducible gene expression, without significant toxicity. *Blood* 105, 489-495 (2005)

90. D. E. Speiser, D. Lienard, N. Rufer, V. Rubio-Godoy, D. Rimoldi, F. Lejeune, A. M. Krieg, J.-C. Cerottini and P. Romero: Rapid and strong human CD8+ T cell responses to vaccination with peptide, IFA, and CpG oligodeoxynucleotide 7909. *J. Clin. Invest.* 115, 739-746 (2005)

91. J. Banchereau and A. K. Palucka: Dendritic cells as therapeutic vaccines against cancer. *Nat Rev Immunol* 5, 296-306 (2005)

92. P. J. Tacken, I. J. M. de Vries, R. Torensma and C. G. Figdor: Dendritic-cell immunotherapy: from *ex vivo* loading to *in vivo* targeting. *Nat Rev Immunol* 7, 790 (2007)

Abbreviations: APC: antigen-presenting cell, DC: dendritic cell, CLP: common lymphoid progenitor, CMP: common myeloid progenitor, GITR: glucocorticoid-induced tumor necrosis factor receptor, GM-CSF: granulocyte-macrophage colony stimulating-factor, ICOS: inducible costimulator, IFN: interferon, IL: interleukin, LPS: lipopolysaccharide, M-CSF: macrophage-colony stimulating factor, mDC: myeloid dendritic cell, ODN: oligodeoxynucleotide, pDC: plasmacytoid dendritic cell, PG: prostaglandin, TGF: transforming growth factor, Th: T helper, TLR: Toll-like receptor, TNF: tumor necrosis factor, TSLP: thymic stromal lymphopoietin

Key Words: Dendritic cells, Innate immunity, Adaptive immunity, T cells, Toll-like receptor, Interferon, Th1, Th2, Immunotherapy, Review

Send correspondence to: Norimitsu Kadowaki, Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, 54 Shogoin Kawara-cho, Sakyo-ku, Kyoto 606-8507, Japan, Tel: 81-75-751-4964, Fax: 81-75-751-4963, E-mail: kadowaki@kuhp.kyoto-u.ac.jp

http://www.bioscience.org/current/vol14.htm