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CONTROL OF MYELOID DENDRITIC CELL DIFFERENTIATION AND FUNCTION BY CD1D-RESTRICTED (NK) T CELLS

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1. ABSTRACT

While regulating a wide variety of immunologic responses, the precise immunologic functions of CD1d-restricted (NK) T cells are not well defined. Notably, *In vitro* activation of human NK T cell clones results in the secretion of multiple cytokines important for the recruitment and differentiation of myeloid dendritic cells (DC). Once differentiated, these DC strongly activate NK T cells. In humans, CD1d is expressed by myeloid DC and on tumor cells of this lineage. Another specialized myeloid antigen presenting cell, the epithelioid histiocyte seen in granulomatous inflammation, also expresses CD1d. Because myeloid DC are important regulators of Th1/Th2 T cell responses, cross talk between human NK T cells and myeloid DC would be expected to have significant impact on many immune responses. Consistent with this hypothesis, NK T cells are required for myeloid DC-controlled antitumor responses in mice, and regulate diabetes in nonobese diabetic (NOD) mouse by locally controlling the frequency and function of DC subsets. Thus, regulation of myeloid DC by NK T cells controls both the transition from innate to adaptive immunity and the Th-phenotype of subsequent T cell responses.

2. INTRODUCTION

CD1d-restricted (NK) T cells are thought to regulate an extremely diverse set of immunologic responses (1) (2) (3) (4) (5) (6). Despite the functional importance of NK T cells in these responses, their mechanism of action has remained enigmatic. Human $CD161^+$ $V24aJaQ^+$ T cells and the murine counterparts, $CD161^+$ $Va14Ja281^+$ T cells, are activated specifically by the non-polymorphic class Ib molecule CD1d through presentation of a glycolipid antigen (7) (8) (9) (10) (11). Murine NK T cells were first suggested to play an important role in initiating Th2 responses through the burst production of IL-4 on activation (12). However, an absolute requirement for CD1d-restricted T cells in the generation of Th2 responses has been excluded by the observation that CD1d knockout mice retain the capacity to generate antigen-specific Th2 responses (13) (14).

Dysfunction and/or diminished frequency of CD1d-restricted T cells clearly correlates with the development of autoimmunity, in particular autoimmune diabetes mellitus, in both rodents and humans(15) (16) (17) (18) (19) (20). For example, CD1d-restricted T cells lines derived from the spleens of non-obese diabetic (NOD) mice are markedly defective in cytokine secretion (17). The importance of CD1d-restricted T cells in preventing diabetes is directly demonstrated by experiments employing either passive transfer of NK T cells into NOD mice or generation of transgenic NOD mice expressing the TCR Va14Ja281 a-chain(21). However, neither passive transfer of CD1d-restricted T cells nor introduction of the Va14Ja281 transgene suffices for full protection of NOD mice from diabetes, highlighting the involvement of additional, unknown factors.

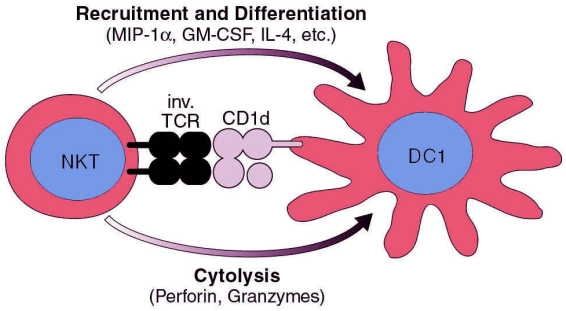

Figure 1. A model demonstrating the interaction of CD1d-restricted T cells with myeloid dendritic cells. Activation of invariant Va24JaQ T cells results in the secretion of cytokines and chemokines important for myeloid dendritic cell recruitment and activation. In addition, important cell surface co-stimulatory molecules are also expressed. During myeloid dendritic cell maturation, CD1d is upregulated and activates CD1d-restricted T cells. In addition to the secretion of cytokines and chemokines, activated Va24JaQ T cells upregulate perforin, granzyme B, and Granulysin. The CD1d-dependent secretion of these molecules then results in the lysis of myeloid dendritic cells.

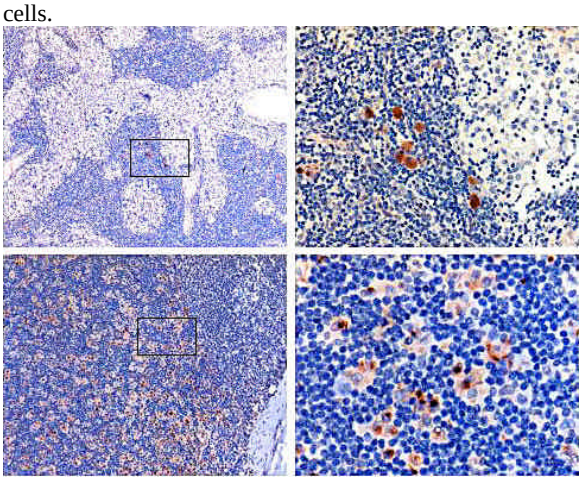

Figure 2. Immunoperoxidase staining of CD1d on paracortical dendritic cells in a reactive lymph node. A monoclonal antibody against CD1d (clone Nor3.2) was used as previously described (11). The upper panels show CD1d expression in paracortical dendritic cells but not in sinus histiocytes. The lower panel shows numerous CD1d positive dendritic cells within the paracortex of a lymph node.

CD1d-restricted T cells are critical for an array of specialized tasks including initiation of antigen specific tolerance, control of certain types of viral infections, maintenance of the gravid state, and tumor surveillance (3) (5) (6) (22). Initial studies have addressed the various roles of NK T cells in immune response regulation--both normal and pathological. More recent studies have begun to characterize these cells at the molecular level. Along these lines, we are applying transcriptional profiling with high-density oligonucleotide arrays. Presumably, identification of gene classes activated in this cell type should predict its specific function and elucidate disease-related dysfunction in key regulatory circuits. Our results show that activation of NK T cell clones by anti-CD3 results in upregulation of transcripts critical for the recruitment and activation of myeloid dendritic cells (DC) (e.g. GM-CSF, IL-4, IL-13, IFN-g, LT-β, TNF-a, MIP-1a, MIP-1b, CD40L, 4-1BB, and TRAIL,) (20) (23) (24). Accordingly, the remainder of this review will discuss the control of myeloid dendritic cell differentiation by NK T cells, focusing on autoimmune diabetes and tumor surveillance model systems.

3. FUNCTIONAL CONSEQUENCES OF CD1D EXPRESSION BY HUMAN MYELOID DC

3.1 *In vitro* cross talk between human myeloid DC expressing CD1d and NK T cells

In vitro cell culture experiments have validated the prediction that NK T cells express a panel of genes important for induction of differentiation in human myeloid DC (11) (20) (23). Peripheral blood monocytes express low levels of CD1d, which is promptly lost on culture *In vitro* (25) (26). Mature myeloid DC derived from peripheral blood monocytes demonstrate reacquisition of surface CD1d expression; in contrast, *In vitro* activated B and T cells do not express CD1d (25). Myeloid DC activate human NK T cells, which may in turn target the myeloid DC for lysis(23). Because human myeloid DC produce IL-12 and control Th1 differentiation (27) (28), their elimination by by cytolytic NK T cells could provide a means for limiting generation of Th1 T cells, thereby regulating the Th1/Th2 balance in an immune response (Figure 1). Moreover, myeloid DC induced expansion of human NK T cells (particularly cord blood derived cells) preferentially biases them toward the secretion of IFN-g (29). Notably, NK T cell clones derived from human patients with type 1 diabetes and NK T cell lines from non-obese diabetic (NOD) mice are functionally defective and most likely impaired in activating myeloid DC (17) (19) (20). In addition myeloid DC in human and murine models of autoimmune diabetes are immature and also functionally defective (30) (31). Thus, there appears to a significant role for NK T cell-DC cross talk in the shaping of both NK T cell and DC immune responses.

3.2. *In vivo* expression of CD1d on human myeloid antigen presenting cells

Immunohistochemical analysis of human lymph nodes has comfirmed CD1d expression on DC in the paracortical T cell zones (Figure 2) (23) (26). As expected these DC also express CD1a and S100 but not CD68. CD1d is not expressed by follicular DC or follicle tingible body macrophages and is largely absent from sinus histiocytes. To define further the scope of expression in histiocytic/dendritic populations, CD1d has been examined in a range of reactive and neoplastic histiocytic disorders (J. Seibel, S.B,Wilson & F. K. Racke, unpublished data). CD1d is consistently expressed by epithelioid histiocytes in various granulomatous reactions including mycobacterial infection (32), sarcoidosis, and tumor-associated granulomata. In some cases of AIDS-associated mycobacterial infection, CD1d staining highlighted histiocytes even in the absence of granuloma formation.

	B6	CD1d (-/-)	B6	Jα281 (-/-)
Tumor	4	19	1	9
No Tumor	18	2	9	3

Figure 3. Vaccination with irradiated, GM-CSF secreting B16 melanoma cells is abrogated in CD1d deficient mice. A). Female C57Bl/6 wild type, CD1d deficient or Ja281 deficient mice were immunized subcutaneously on the abdomen with $5x10^5$ irradiated, GM-CSF secreting B16 cells and one week later challenged subcutaneously on the back with $1x10^6$ wild type B16 cells. Animals were considered tumor free if they did not develop tumors during 60 days of observation. The CD1d deficient mice were significantly more susceptible to wild type tumor challenge than controls ($\chi 2$, $p=2 \times 10^{-6}$). Vaccination with irradiated, wild type B16 tumor cells failed to elicit protective immunity in either strain (not shown).

In a mouse model system, NK T cells are required for granuloma formation in response to injected mycobacterial cell wall antigens (33). We have found intense CD1d staining on the palisading histiocytes of these *M. t.b.* granulomas; in addition, the surrounding lymphocytes react with antibodies to the Va24 TCR chain and to the TCR a-chain CDR3 loop found on human invariant NK T cells (F. Racke & S.B. Wilson, unpublished).

CD1d is also consistently expressed in Langerhans cell histiocytic (LCH) lesions and interdigitating dendritic cell tumors (IDCS). Langerhans cell histiocytosis is a rare clonal disorder of cells similar to normal cutaneous Langerhans cells, characterized by coexpression of S100 and CD1a. Normal Langerhans cells are thought to be hematopoietic stem cell derived. While normal Langerhans cells lack myeloid antigen expression, the tumor cells of Langerhans cell histiocytosis usually express myeloid antigens, suggesting a possible relationship between LCH and myeloid DCs. Interdigitating dendritic cell tumors, probably derived from paracortical DCs, are even rarer than LCH. In contrast to LCH and IDCS, myeloid leukemias show no significant expression of CD1d. Therefore, CD1d expression, both in benign and neoplastic conditions, suggests a specific cell lineage derived from myeloid antigen presenting cells. Notably, a major clinical sequela in patients with LCH is tissue damage due to inflammatory reactions to the neoplastic Langerhans cells. The high level of expression of CD1d on these cells makes them potentially amenable to novel immuno-therapeutic strategies targeting the CD1d-NKT cell axis.

4. REGULATION OF MURINE MYELOID DC FUNCTION BY NK T CELLS

4.1 Cross talk between NK T cells and myeloid DC is critical for anti-tumor immunity

In mice, antibody-mediated depletion of NK T cells or knockout by gene targeting of Va14Ja281 highlights the critical roles of NK T cells in the anti-tumor effects of low dose interleukin-12 treatment (4) (34) (35) (36) (37). Additional studies have shown that $Va14Ja281^+$ T cells are also required for protection against tumor development induced by chemical carcinogens (6). These anti-tumor functions are markedly augmented by a-galactosylceramide (a-GalCer), an activating glycolipid antigen presented by CD1d, through a mechanism involving IL-12 production by DC (10) (38) (39). The anti-tumor activities of Va14Ja281 T cells include perforin-dependent NK-like cytotoxicity, IFN-g production, and stimulation of CD8 positive T lymphocytes (8) (40).

The recognition that DCs play crucial roles in priming antigen-specific responses has led to the design of numerous protocols exploiting these cells for the induction of anti-tumor immunity (41). Either the ex vivo manipulation of DCs or the *In vivo* administration of cytokines activating DCs can enhance tumor rejection in model systems (42) (43). For example, vaccination with irradiated tumor cells engineered to secrete GM-CSF stimulates potent, specific, and long-lasting anti-tumor immunity in multiple murine models (44). Although both GM-CSF and Flt3-ligand (FL) induce equivalent expansion of DCs, GM-CSF secreting tumor cells function as a more potent tumor vaccine than do irradiated, FL secreting tumor cells (45). Notably, the superior efficacy of the GM-CSF vaccine is associated with high level CD1d expression on CD8a-, CD11c+ DCs.

While DCs are required for the efficient priming of antigen-specific lymphocyte responses, T cells in turn are required for optimal DC maturation (46) (47). The transcriptional profile of activated NK T cells, namely the production of factors promoting myeloid DC maturation, together with the high level CD1d expression on GM-CSF stimulated DCs, suggests a role for the NK T cell-myeloid DC axis in tumor immunity. We have found that the anti-tumor response stimulated by vaccination with irradiated, GM-CSF secreting tumor cells is abrogated both in CD1d -/- mice and in Ja281 -/- mice, confirming a requirement for CD1d-restricted NK T cells and presumably myeloid DC in this anti-tumor response (Figure 3).

4.2 Regulation of autoimmune diabetes in NOD mice by NK T cell and DC

In vivo activation of NK T cells with the ligand a-GalCer has a significant impact on the course of diabetes development in NOD mice with preexisting insulitis. In particular, weekly injections of a-GalCer significantly reduce and delay development of diabetes; by contrast, similar therapy has no effect in CD1d -/- mice (Figure 4) (18). Injection of mice with a-GalCer is known to result in burst secretion of IL-4 and IFN-g by NK T cells (8). Chronic administration of a-GalCer induces in antigen-specific T cells a Th2 bias that is associated with augmented IL-4 secretion by NK T cells (48). IL-4 production by NK T cells may promote this Th2 transition, but Th2-like responses can also continue unabated in the absence of NK T cells (13) (49).

DC-derived IL-12 appears to influence diabetes development in the NOD model. *In vitro* pre-activation

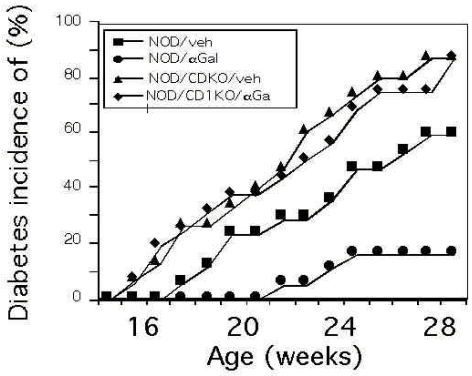

Figure 4. a-GalCer prevents diabetes only in wild type NOD mice. Starting at 3-4 weeks of age, Female NOD and NOD/CD1d KO mice were injected with a-GalCer or vehicle on a weekly basis. Diabetes was assessed by monitoring blood glucose levels every week, and mice with two consecutive blood glucose measurement greater than 250 mg/dl were considered diabetic (RR=0.46, p=0.002 a-GalCer NOD wt; RR=1.7, p=0.006 NOD/CD1dKO) NOD/veh N=17, NOD/a-GalCer N=18, NOD/CD1d KO veh, N=15, NOD/CD1dKO a-GalCer N=16.

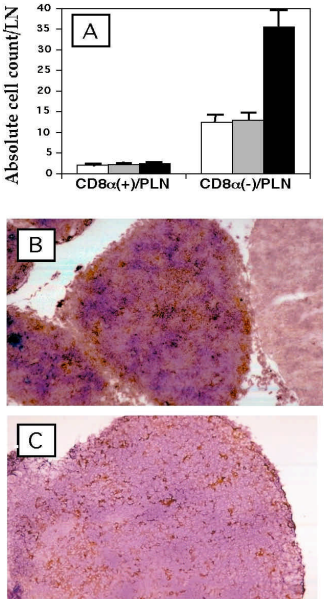

Figure 5. Treatment with a-GalCer results in the preferential accumulation of myeloid CD8a-/CD11c+ dendritic cells in the lymph nodes draining the pancreas. A. The total number of CD8a+/CD11c+ (lymphoid) and CD8a-/CD11c+ (myeloid) DC in pancreatic and inguinal lymph nodes were determined by FACS 10 days after mice were treated with vehicle, control a-ManCer, or a-GalCer i.p. on days 0 and 4 (n=6/treatment, for a total of 6 experiments). Treatment with a-GalCer resulted in significantly more myeloid DC than lymphoid in the pancreatic lymph node (p=0.005, students t). No CD11c staining was observed on T, B, or NK cells.(data not shown) B & C. Immunohistochemical staining for CD11c+ cells (brown deposits) in representative lymph nodes from mice treated with a-GalCer (B), or vehicle (C).

with IL-12 of CD1d-restricted T cell lines derived from the spleens of NOD mice enhances their capacity to protect naive recipient NOD mice from diabetes (17). Despite the enhanced protection conferred by CD1d-restricted T cells treated with IL-12, these cells are markedly hyporesponsive to IL-12 stimulation. Defective activation is a hallmark of CD1d-restricted T cells derived from humans and mice with autoimmune diabetes (20) (50). Therefore, a-GalCer therapy may act to overcome the hyporesponsiveness in NOD mice of CD1d-restricted T cells and permit them to exert their critical immunoregulatory functions.

Recently, a genetic basis has been established for the capacity of self-reactive T cells to cause autoimmunity through induction of IL-12 secretion by antigen presenting cells (APCs) (51). IL-12, when administered chronically, accelerates diabetes development in NOD mice by driving the development of Th1-biased autoreactive T cells (52). Activated dendritic cells are thought to be a major source of endogenous IL-12 (53) (54) (55). These dendritic cells infiltrate the islets early in the inflammatory response and contribute both to the initiation and maintenance of insulitis (56) (57) (58) (59). Paradoxically, passive transfer of mature myeloid dendritic cells may actually prevent diabetes development in NOD mice (18, 60) (61) (62). In addition, DC also appear to play a role in generation of peripheral tolerance in transgenic mice expressing ovalbumin or influenza hemagglutinin in pancreatic beta cells (63) (64) (65).

The recent identification of dendritic cell subsets that differentially regulate T cell responses may help resolve the paradoxical roles of dendritic cells in diabetes development. In the mouse, but not in humans, a CD11c+, CD11b-, CD8a+ dendritic cell subset is the major DC source of IL-12. This DC subset expresses higher levels of CD1d than the myeloid CD11c+, CD11b+, CD8a- and CD11c+, CD11b-, CD8a- populations (55) (66). The apparent functions of the CD8a+ DC subset are to promote Th1-biased immune responses, to prevent development of peripheral tolerance, and to cross-prime cytotoxic T cells *In vivo* (53) (54) (67) (68). Conversely, the two CD8a- DC subsets appear to promote Th0 or Th2-like responses and not cross-prime CTL (54) (67) (68) (69) (70). Importantly, the dendritic cell subsets that transfer protection from diabetes to naïve NOD recipients are the two myeloid CD8a- populations(18). These are the same cells whose frequency is augmented in the pancreatic lymph nodes of NOD mice treated with α-GalCer (Figure 5) (18) (60) (61) (62). Thus, in rodents, activation of NK T cells leads to significant migration of CD8a- myeloid DC into the draining pancreatic lymph nodes, resulting in protection from autoimmunity.

5. SUMMARY

CD1d-restricted T cells are important regulators of several different immune responses. Appropriate or inappropriate activation of these T cells has a profound impact on the course of the subsequent immune response. Although the phenotypic spectrum of CD1d-restricted T

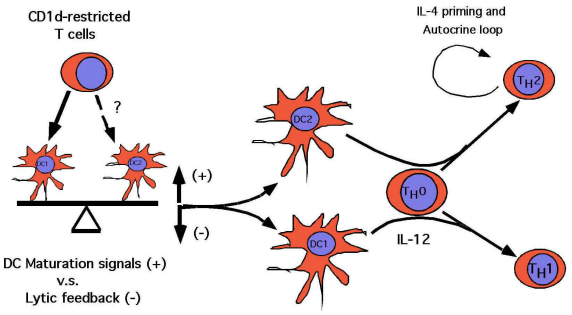

Figure 6. Acivation of CD1d-restricted NK T cells changes regulates immune responses by changing the balance of dendritic cell subsets. Since human myeloid-derived dendritic cells (DC1) and lymphoid-derived dendritic cells (DC2) regulate CD4+ T helper cell responses, the specific lysis of DC1 cells by NK T cells suggests that their immunomodulatory function is not limited to Th2 bias induced by IL-4 secretion. When co-cultured with T cells, DC1 cells secreted high levels of IL-12 and induced T cells with a Th1 phenotype. Co-culture of T cells with DC2 cells induced a marked Th2 response. Thus, the specific lysis of (DC1) cells by NK T cells may serve as a negative feedback mechanism for limiting Th1 T cell responses. In contrast, the secretion of cytokines that would enhance DC2 differentiation, as predicted for murine myeloid DC, would bias responses toward a Th2 like environment. Although there is strong evidence in the mouse that different dendritic cell subsets reciprocally regulate T cell phenotypes and that NK T cells influence DC function, there is no data available on a direct interaction in between murine NK T cells and DC.

cells and the functional consequences of CD1d expression on various APC both remain to be defined, a critical 2-way interaction of these T cells with dendritic cell subsets constitutes a critical regulatory circuit in many of their reported functions (Figure 6).

6. ACKNOWLEDGMENT

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