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Regulation of Mucosal Dendritic Cell Function by Receptor Activator of NF-κB (RANK)/RANK Ligand Interactions: Impact on Tolerance Induction

Eilidh Williamson, Janine M. Bilsborough, and Joanne L. Viney

The mucosal immune system is uniquely equipped to discriminate between potentially invasive pathogens and innocuous food proteins. While the mechanisms responsible for induction of mucosal immunity vs tolerance are not yet fully delineated, recent studies have highlighted mucosal dendritic cells (DC) as being important in determining the fate of orally administered Ag. To further investigate the DC:T cell signals involved in regulating the homeostatic balance between mucosal immunity and tolerance, we have examined the expression and function of the TNFR family member receptor activator of NF-κB (RANK) and its cognate ligand, RANKL, in vitro and in vivo. Our data show that although DC isolated from mucosal lymphoid tissues expressed similar levels of surface RANK compared with DC isolated from peripheral lymphoid tissues, DC from the distinct anatomical sites displayed differential responsiveness to RANK engagement with soluble RANKL. Whereas splenic DC responded to RANKL stimulation with elevated IL-12 p40 mRNA expression, Peyer’s patch DC instead preferentially displayed increased IL-10 mRNA expression. Our data also show that the in vivo functional capacity of mucosal DC can be modulated by RANKL. Treatment with RANKL in vivo at the time of oral administration of soluble OVA enhanced the induction of tolerance in two different mouse models. These studies underscore the functional differences between mucosal and peripheral DC and highlight a novel role for RANK/RANKL interactions during the induction of mucosal immune responses. The Journal of Immunology, 2002, 169: 3606–3612.

The qualitative and quantitative nature of T cell:APC interactions may critically determine the outcome of an immune response. Dendritic cells (DC) are reportedly the most potent immunostimulatory APC and have, until recently, been uniquely associated with the induction of active immune responses (1). Our studies have highlighted an additional functional role for DC in promoting tolerogenic responses in the intestine (2), and subsequent studies by several other groups have demonstrated that DC localized in peripheral lymphoid tissues may also have the capacity to present Ag in a tolerogenic manner (3–5). Despite the revelation that DC can promote both tolerance and active immunity, precisely how and when a DC promotes tolerance vs active immunity is still unclear and has been the subject of much debate (6–9). Studies in our laboratory have demonstrated that intestinal DC naturally promote tolerance, but they have the additional potential to support active intestinal immunity when there are appropriate local inflammatory signals to induce their activation (10). Our current studies have focused on identifying additional, DC-specific signals that may ultimately determine whether an antigenic encounter with a naive T cell promotes tolerance or active immunity.

The structurally related proteins that comprise the TNF and TNF receptor (TNFR) superfamilies mediate a number of important biological activities, ranging from proliferation and differentiation to cell survival and death (11, 12). An integral role for several TNF/TNFR family members in DC biology has recently emerged. TNF and CD40 ligand (CD40L) are both involved in the differentiation of DC from CD34+ progenitor cells (13), and CD40L plays a role in DC survival and can elicit DC cytokine production (14). More recently, a new member of the TNFR family, receptor activator of NF-κB (RANK), and its ligand (RANKL; also known as TNF-related activation-induced cytokine, osteoclast differentiation factor, and osteoprotegrin ligand) have been cloned (15, 16). RANKL expression is restricted to T cells and lymphoid tissues (15, 16). Similarly to CD40L, RANKL is reported to increase the survival of bone marrow-derived DC in vitro by up-regulating Bcl-xL expression (17) and to enhance the ability of DC to stimulate naive T cell proliferation in a mixed lymphocyte reaction (16, 18). However, unlike CD40L, RANKL selectively acts on mature DC, but not B cells (17). Splenic DC have been shown to express RANK (16), and RANK mRNA is highly expressed in the intestine (19), although which cells in the intestine express RANK protein has not yet been determined.

In the present study we confirm that RANK is expressed on DC from the spleen (SPL), and now show that RANK is also expressed at a similar level on DC isolated from mucosal-associated lymphoid tissues (Peyer’s patch (PP) and mesenteric lymph node (MLN)). We have also discovered that engaging the RANK receptor on DC using soluble RANKL selectively increased IL-12 p40 expression in SPL DC as expected, but, surprisingly, did not alter IL-12 p40 expression in PP, MLN, or peripheral lymph node (PLN) DC. In contrast, exposure to RANKL increased the expression of IL-10 mRNA levels in PP DC, although IL-10 levels were not altered in MLN, SPL, or PLN DC. Using two different models of tolerance induced by feeding soluble OVA, the studies presented here highlight a novel functional role, in vivo, for RANK/RANKL interactions during the inductive phase of oral tolerance. We observed that mice treated with RANKL at the time of OVA...
feeding exhibited a more profound degree of tolerance than control untreated animals fed OVA. This was particularly evident in mice fed low doses of OVA, which were ineffective at inducing tolerance in control animals, but induced tolerance in RANKL-treated animals. These studies reveal the functional heterogeneity that exists within APC residing in distinct anatomical locations and provide important information pertinent to the design of immunotherapies or vaccines, particularly those targeted specifically to mucosal surfaces.

Materials and Methods

**Mice**

Female BALB/c mice (6–10 wk of age) were obtained from Taconic Farms (Germantown, NY) and maintained in a specific-pathogen-free facility at Immunex in accordance with approved ethical guidelines. BALB/c DO11.10 OVA TCR transgenic (Tg) mice (20) were bred and maintained in the specific-pathogen-free facility at Immunex.

**Analysis of RANK expression on peripheral and gut-associated lymphoid tissues (GALT) DC using flow cytometry**

SPL, PLN (two inguinal and popliteal lymph nodes per animal), MLN, and PP were removed from groups of five mice that had been injected i.p. daily for 10 days with 10 μg purified CHO-derived human Flt3 ligand (produced and purified at Immunex as previously described [21]), and single-cell suspensions were prepared. Previous studies have shown that Flt3 ligand (Flt3L) expands all DC populations without inducing their activation (2, 22). Cells were incubated for 40 min at 4°C with biotinylated anti-RANK Ab (clone M395; Immunex), together with FITC-labeled anti-CD11c (HL-3, hamster IgG), both used at 10 μg/ml, in 50 μl blocking buffer (PBS containing 10 μg/ml anti-CD16 (BD PharMingen, San Diego, CA), 10% normal goat serum, 2% normal rabbit serum, and 2% normal rat serum). After being washed twice with PBS/2% FBS, cells were incubated with 10 μ/ml PE-labeled streptavidin (10 μg/ml; Molecular Probes, Eugene, OR) for an additional 20 min. Samples were washed three times, resuspended in PBS supplemented with 1% paraformaldehyde, and analyzed on a FACSscan flow cytometer (BD Biosciences, San Jose, CA). At least 30,000 cells were analyzed per sample.

**DC isolation and purification**

Groups of 10 mice were injected daily with 10 μg Flt3L given i.p. for 10 days to expand DC in vivo. The animals were sacrificed; their SPL, PLN (two axillary, brachial, cervical, inguinal, and popliteal lymph nodes per animal), MLN, and PP were removed; and individual tissue types were pooled. Single-cell suspensions were prepared by pressing the excised lymphoid tissues through nylon mesh. The resultant suspension was then spun down, washed, and incubated with anti-mouse CD11c-coated magnetic beads for an additional 20 min. Samples were washed three times, resuspended in PBS supplemented with 1% paraformaldehyde, and analyzed on a FACSscan flow cytometer (BD Biosciences, San Jose, CA). At least 30,000 cells were analyzed per sample.

**Stimulation of DC with RANKL in vitro**

Purified DC (3 × 10^5/well) were cultured for 18 h for RNA studies and for up to 120 h for DC survival studies in 96-well, flat-bottom, tissue culture plates in either complete RPMI (RPMI supplemented with 10% FBS, penicillin/streptomycin, 1-glutamine, and 2-ME) or complete RPMI containing 10 μg/ml soluble murine RANKL-isolecine zipper fusion protein, produced and purified at Immunex, in a total volume of 200 μl.

**Isolation of RNA from DC cultures**

RNA was prepared following homogenization of DC in guanidinium isothiocyanate buffer (4.5 M guanidinium isothiocyanate, 50 mM sodium citrate, 0.5% (w/v) sodium sarcosyl) containing 2% 2-ME (Life Technologies, Gaithersburg, MD). RNA was then extracted using acid phenol, as described previously (23). RNA samples were subsequently treated with DNase (DNA-free; Ambion, Austin, TX) to eliminate contaminating genomic DNA, and the resulting purified RNA samples were reverse transcribed to cDNA with random hexamers according to the manufacturer’s instructions (TaqMan Reverse Transcription Reagents; PerkinElmer, Boston, MA).

**Primers and fluorogenic probes for real-time PCR**

Oligonucleotide primers and TaqMan probes for murine IL-10, IL-12 p40, and RANK were purchased from PerkinElmer. Primers and probes for murine β-actin and porphobilinogen deaminase (PBGD) were designed using Primer Express software and were synthesized by PerkinElmer. Forward primer, reverse primer, and probe sequences (5'–3') for β-actin (forward primer, TCTTCTGTCGCGTGCACCTC; reverse primer, ACCAGCGCACGGATATGC; probe, CCACCGACAGTTGACATG) and PBGD (forward primer, CTGCGCTACACGCCATCAGG; reverse primer, CTTCCTCAGGCGTCAATACT; probe, TGCCGACCGCTGTCGCA) are shown in parentheses.

**Quantification of gene expression using real-time PCR**

Levels of RANK, IL-10, and IL-12 p40 mRNA were determined by Taq-Man PCR analysis. Sequence-specific amplification of the genes of interest was detected by an increased fluorescent signal of FAM (reporter dye) during amplification. Murine β-actin and PBGD were used as endogenous controls in a multiplex TaqMan PCR (TaqMan Universal PCR Master Mix; PE Biosystems, Foster City, CA) to allow for relative mRNA quantification. Cytokine mRNA levels are presented as the mean ± SD of the yields achieved in gene expression observed in triplicate wells of RANKL-treated DC relative to untreated DC and are representative of data achieved in three separate, independent experiments.

**Assessment of oral tolerance in adoptive transfer mice**

For adoptive transfer of OVA TCR Tg T cells, BALB/c mice were injected i.v. with 2.5 × 10^7 clonotypic TCR (CD4(+) KJ1.26) Tg cells from DO11.10 mice, as previously described (2). Groups of three BALB/c mice were then treated with PBS or 10 μg/ml RANKL-isolecine zipper fusion protein, given i.p. at −18, −3, and 0 h relative to OVA feeding. Mice were fed a single dose of 25 or 0.5 mg OVA in 0.2 ml saline, or saline only, 2 days after transfer of Tg T cells and were immunized s.c. in the footpad with 100 μg OVA in RIBI adjuvant (RIBI Immunochemicals, Hamilton, MT) 5 days later. After another 4 days, draining PLN were removed, and the Ag-specific proliferative capacity of the lymph node cells was examined by culturing the cells in complete RPMI medium at a density of 2 × 10^5 cells/well for 48–96 h. Cultures were performed in triplicate in 96-well flat-bottom plates in a total volume of 200 μl either alone or in the presence of 1 or 0.3 mg/ml OVA. Proliferation was assessed by addition of 1 μCi/well [3H]thymidine (Amersham, Little Chalfont, U.K.) 18 h before harvesting. The amount of 3H thymidine incorporation by each test sample was measured using a Matrix-96 cell harvester (Innotech, Lansing, MI) and a direct beta counter (Packard, Meridian, CT). In addition, the proportion of Tg T cells in adoptively transferred mice was determined by FACS analysis. Cells were incubated with mAb KJ1.26 FITC, which detects the clonotypic Tg TCR, and anti-CD4 PE (BD PharMingen) in 50 μl blocking buffer containing 10 μg/ml anti-CD16 (BD PharMingen), 10% normal goat serum, and 1% normal mouse serum and were analyzed on a FACSscan (BD Biosciences) as described above.

**Induction and assessment of conventional oral tolerance**

BALB/c mice (six mice per group) were injected i.p. with PBS or 10 μg/ml RANKL, given at −18, −3, and 0 h relative to OVA feeding (given as a single dose of 0.5, 0.25 mg OVA in 0.2 ml saline by gavage). Ten days after OVA feeding, mice were immunized s.c. into the footpad with 100 μg OVA in RIBI adjuvant (RIBI Immunochemicals). Twenty-one days after immunization, mice were assayed for systemic delayed-type hypersensitivity (DTH) responses by measuring the increase in footpad thickness 24 h after challenge with 100 μg heat-aggregated OVA in 50 μl saline as previously
described above. The mice were then sacrificed, and the draining PLN were removed for assessment of in vitro Ag-specific proliferative capability, as described above.

**Results**

**RANK is expressed at similar levels on SPL, PLN, MLN, and PP DC**

To determine expression levels of RANK on DC isolated from peripheral and mucosal lymphoid tissues, DC were purified from SPL, PLN, MLN, and PP of Flt3L-treated mice. RNA was then isolated, and levels of RANK mRNA were measured using TaqMan Real-Time PCR. As shown in Fig. 1A, RANK mRNA was expressed at similar levels in DC isolated from each of these tissues. To confirm these findings at the protein level, we also conducted flow cytometric analysis to determine RANK expression on gated CD11c+ SPL, MLN, and PP DC. Again, RANK was detected at similar levels on each of these DC populations regardless of the anatomical site (Fig. 1B). Although the level of staining was low, the specificity of the FACS staining was confirmed by cold competition with unlabeled anti-RANK mAb (data not shown).

**Effect of RANKL on purified peripheral and mucosal DC**

Given the similar levels of RANK expression on DC from peripheral and mucosal lymphoid tissues, we next examined how DC isolated from each of these different anatomical compartments responded following RANK engagement. To examine the effect of RANK engagement on cytokine induction by each of these DC populations, DC were again purified from the SPL, PLN, MLN, and PP of Flt3L-treated mice and were incubated in the presence or the absence of soluble murine RANKL for 18 h. RNA was then isolated from the DC cultures, and levels of cytokine mRNA were measured using TaqMan real-time PCR.

Analysis of cytokine mRNA levels indicated that DC isolated from SPL, PLN, MLN, and PP exhibited notably disparate gene expression profiles for IL-10 and IL-12 p40 following RANKL stimulation (Fig. 2). While PLN, MLN, and PP-derived DC did not show an appreciable difference in the levels of IL-12 p40 mRNA after exposure to RANKL in vitro, expression of IL-12 p40 mRNA was up-regulated several-fold in SPL DC. In contrast, whereas IL-10 mRNA expression was not significantly altered in SPL, MLN, or PLN DC following RANKL treatment, elevated IL-10 mRNA expression was observed in RANKL-treated PP DC. Interestingly, IL-18 mRNA expression was consistently down-regulated in SPL, MLN, and PP DC, but not in PLN DC, following RANKL treatment in vitro (data not shown). Taken together, these data suggest that DC isolated from different tissues behave differently in response to stimulation with RANKL.

RANKL treatment of bone marrow-derived DC in vitro has been reported to induce cell aggregation and enhance survival. We tested whether RANKL treatment would prolong the life span of the primary peripheral and mucosal DC cultured in vitro in this study. RANKL treatment induced DC aggregation (Fig. 3A), but no increase in the survival of primary peripheral and mucosal DC was observed (Fig. 3B).

![FIGURE 1](http://www.jimmunol.org/)

**FIGURE 1.** Expression of RANK mRNA (A) and protein (B) in DC isolated from peripheral and mucosal lymphoid tissues. A. Levels of RANK mRNA were determined in CD11c+ DC purified from SPL, MLN, PP, and PLN of Flt3L-treated mice using TaqMan real-time PCR analysis. RANK mRNA levels are presented as Δ cycle threshold (Δ CT), i.e., the cycle number at which the amplification of the gene lies within the exponential phase of amplification, normalized to the internal housekeeping gene. Data shown are representative of results achieved in three separate, independent experiments. B. RANK protein expression was determined on freshly isolated cells from SPL, MLN, and PP of Flt3L-treated mice using flow cytometry. The histograms represent the levels of RANK on gated CD11c+ DC from each of the indicated tissues. Filled histograms show isotype control Ab staining. Data are representative of four independent experiments.

![FIGURE 2](http://www.jimmunol.org/)

**FIGURE 2.** Differential effect of RANKL on cytokine gene expression in purified peripheral and mucosal DC. Purified DC from SPL, MLN, PP, and PLN were cultured in the presence or the absence of soluble RANKL for 18 h, after which the RNA was isolated, and the levels of IL-10 and IL-12 p40 were determined by TaqMan PCR analysis. Murine PBGD and β-actin were used as endogenous controls to allow for relative mRNA quantification. Cytokine mRNA levels are shown as the mean (±1 SD) fold increase in gene expression observed in triplicate wells of RANKL-stimulated DC relative to unstimulated DC and are representative of three separate independent experiments.
Treatment with RANKL in vivo enhances oral tolerance induction in an adoptive transfer model

We have recently highlighted a central role for DC as APC for oral tolerance induction (2, 10) and identified GALT as the inductive site for oral tolerance (25). Since we found that RANKL promoted increased expression of mRNA for the immunosuppressive cytokine IL-10 in PP DC, but not in DC from peripheral lymphoid tissues, we next wanted to determine whether RANK engagement in vivo could modulate the outcome of an immune response to orally administered soluble OVA.

We first used an adoptive transfer model in which OVA-specific Tg T cells from D011.10 mice are transferred into normal unirradiated BALB/c recipients and detected using the anti-clonotypic TCR mAb KJ1-26 (26). Previous studies have shown that the number of OVA-specific Tg T cells detectable in draining lymph node (DLN) of adoptively transferred mice is dramatically increased following immunization (26). In contrast, when Ag is administered in a tolerogenic manner such as by the oral route (27), before immunization, the number of Tg T cells detectable in the DLN is decreased, and these cells exhibit a reduced Ag-specific proliferative capacity in vitro. As we have previously observed (2), feeding OVA induced a dose-dependent reduction in the percentage (Fig. 4A), absolute number (Fig. 4B), and proliferative capacity (Fig. 4C) of Tg T cells recovered from the DLN of adoptively transferred mice fed OVA or saline, immunized 5 days later, and analyzed after an additional 4 days. CD4+ Tg T cell numbers in the DLN of adoptively transferred mice were quantitated by FACS staining using the clonotypic mAb KJ1-26 and anti-CD4. Saline- and RANKL-injected mice fed 25 mg OVA had a reduced frequency (A) and lower absolute numbers (B) of CD4+ Tg T cells than equivalent saline-fed mice (*, p < 0.01). Proliferative capacity of the Tg T cells was assessed by [3H]thymidine incorporation into DNA in an 18-h culture period. DLN cells from control (saline-injected) mice fed 25 mg OVA had significantly reduced Ag-specific proliferation compared with control mice fed saline (+, p < 0.01), whereas cells from control mice fed 0.5 mg OVA showed normal proliferative responses. Cells from RANKL-treated mice fed either low (0.5 mg) or high (25 mg) OVA showed significantly reduced proliferation compared with cells from RANKL-treated mice fed saline (#, p < 0.001). Notably, the proliferative response of cells from RANKL-treated mice fed low dose OVA was significantly reduced compared with the response of cells from saline-treated mice fed low dose OVA (¶, p < 0.001). The data are reported as the mean cpm ± 1 SD of triplicate wells and are representative of three separate experiments.
dose-dependent reduction in the percentage (Fig. 4A) and absolute number (Fig. 4B) of Tg T cells compared with that seen in OVA-fed mice that did not receive RANKL. However, the functional capacity of DLN cells from RANKL-treated, OVA-fed mice was quite distinct from that of cells from saline-treated, OVA-fed mice. This was particularly evident at low doses of OVA, which were insufficient to induce tolerance in saline-injected mice. Accordingly, DLN cells from RANKL-treated mice fed low doses of OVA showed dramatically reduced OVA-specific proliferation in vitro compared with cells from saline-injected, OVA-fed mice (Fig. 4C). The markedly reduced proliferation of DLN cells from RANKL-treated mice fed low dose OVA resembled the diminished functional capacity of cells from saline-injected mice fed high dose OVA (Fig. 4C). RANKL treatment had little effect on the low levels of proliferation observed at feeding a high dose OVA (Fig. 4C), most likely because these levels were already maximally suppressed.

**Treatment with RANKL in vivo enhances tolerance in a conventional model of oral tolerance induction**

The above findings led us to examine whether RANKL might also modulate mucosal tolerance using a well-characterized, conventional model of oral tolerance induction. Control (saline-injected) mice fed soluble OVA before immunization with OVA plus adjuvant showed a dose-dependent reduction in DTH and Ag-specific in vitro proliferative responses (Fig. 5), as we have previously described (2). Thus, while the DTH and proliferative responses of control mice fed low dose OVA (0.5 mg) were essentially normal (Fig. 5), control mice fed high dose OVA (25 mg), displayed significantly suppressed in vivo and in vitro T cell responses (Fig. 5).

The pattern of tolerance induced by feeding OVA to RANKL-treated mice, was however, quite distinct from that observed in control OVA-fed mice. RANKL-treated mice fed low dose OVA showed markedly suppressed DTH and in vitro proliferative responses compared with equivalent control mice fed this dose of Ag (Fig. 5), such that RANKL-treated mice fed low dose OVA resembled control mice fed high dose OVA. RANKL had little effect on the profound degree of tolerance exhibited in mice fed high dose OVA. As such, RANKL-treated mice fed high dose OVA exhibited the same markedly suppressed responses as saline-injected controls fed high dose OVA (Fig. 5). Again, the fact that RANKL had little effect on the suppressed responses observed in mice fed high dose OVA may be attributed to the fact that these levels were already maximally suppressed.

**Discussion**

The data presented here indicate that, similar to DC isolated from SPL or PLN, DC isolated from MLN and PP expressed RANK mRNA and low levels of surface RANK protein. However, despite similar levels of surface RANK expression, RANK engagement using soluble RANKL had a distinct effect on the profile of cytokine genes expressed in DC isolated from each of these tissues. Whereas exposure to RANKL in vitro selectively up-regulated IL-12 p40 mRNA expression in SPL DC, an unexpected observation was that IL-10 mRNA, rather than IL-12 p40 mRNA, was selectively increased in RANKL-treated PP DC. The studies presented here also indicate that the functional capacity of mucosal DC in vivo can be modulated by RANKL. Treatment with RANKL around the time of oral administration of soluble OVA enhanced the induction of tolerance in two different mouse models. Most notably, tolerance could be induced in RANKL-treated mice using low doses of Ag that were ineffective in control animals. Our data provide further support for the hypothesis that DC play a central role in Ag presentation at mucosal surfaces such as the gut and highlight a novel role for RANK/RANKL interactions during the induction of mucosal immune responses. These studies indicate that all DC are not created equally, and that DC from different anatomical sites behave differently in response to the same stimulus.

Previous studies that have examined the effect of RANKL on DC have indicated that RANKL can promote the survival of bone marrow-derived DC (18). Unlike bone marrow-derived DC, peripheral and mucosal DC do not appear to exhibit prolonged survival in response to RANKL treatment. Previous studies examining the effect of RANKL on DC have also shown that RANKL...
can enhance the allostimulatory capacity of splenic DC in MLR (16). Since RANKL does not increase levels of classical surface costimulatory molecules such as CD80, CD86, and ICAM-1 or MHC class I on splenic DC (17), the enhanced allostimulatory effects of RANKL on DC function are unlikely to be attributed to RANKL-induced alterations in DC surface phenotype. Rather, RANKL treatment of splenic DC has been shown to induce IL-12 expression (17). We therefore chose to focus our current studies on examining whether RANKL functions primarily to modulate this aspect of DC function, namely, cytokine expression. Analysis of cytokine mRNA levels by TaqMan real-time PCR revealed that while RANKL exposure increased IL-12 p40 mRNA expression in splenic DC, it had no effect on induction of IL-12 p40 mRNA in DC isolated from PLN, MLN, or PP. In contrast, RANKL selectively up-regulated IL-10 mRNA expression in PP DC, but not in splenic, PLN, or MLN DC. The fact that RANKL increased IL-10 mRNA in PP, but not MLN, DC points to differences in RANKL responsiveness within the mucosal DC compartment itself.

A topic of debate in the field of DC biology is the question of whether DC from different tissues behave similarly, or whether they are phenotypically and functionally distinct. Recent studies have suggested that PP and splenic DC may differ in terms of their cytokine profile and ability to induce the differentiation of distinct Th cell subsets (28). Our data suggest that DC from different anatomical sites may show even greater differences than anticipated. We found that although SPL, PLN, MLN, and PP DC exhibited comparable levels of surface RANK expression and similar survival patterns, these DC clearly displayed differential responsiveness to RANK engagement. While SPL DC responded to RANKL stimulation with elevated IL-12 p40 mRNA expression, PP DC instead preferentially displayed increased IL-10 mRNA expression. Previous studies performed using murine bone marrow-derived DC have demonstrated that RANK engagement using a recombinant TNF-related, activation-induced cytokine fusion protein also promoted increased IL-12 p40, but not IL-10, mRNA expression (29), suggesting that PP DC may be uniquely disposed to IL-10 production. Interestingly, it has recently been demonstrated that DC at another mucosal surface, the respiratory tract, may also mediate tolerance through induction of IL-10 (30).

The data presented here show that DC isolated from different sites respond differently to the same stimulus, in this case delivered by RANKL. This raises the question of whether DC localized in different tissues are congenitally different, or whether they become specialized in response to extrinsic factors in the local tissue microenvironment. To date it has not been possible to distinguish mucosal vs peripheral DC in terms of surface phenotype. However, this may simply reflect the lack of appropriate reagents available to make this distinction and is an important area for future studies. What relevance is there to the observed differential responsiveness of DC localized in GALT vs SPL? Studies in our own and other laboratories have shown that the inductive events leading to oral tolerance probably occur in the local GALT environment very early after Ag feeding (25). Furthermore, our studies support the idea that DC at these mucosal sites are involved in the presentation of orally encountered Ag to local T cell populations (2, 10, 25). In particular, we have observed that MLN and PP are the major sites of T cell reactivity following oral administration of Ag, and that the initial events associated with the induction of oral tolerance are heightened when increased numbers of DC are present at these sites (2). The present data show that DC localized in mucosal tissues respond to RANKL stimulation by up-regulating the expression of the suppressive cytokine, IL-10. This is likely to create a tolerogenic environment, conducive to promoting the T cell hyporesponsiveness normally observed in response to peripherally administered soluble proteins. In agreement with this, our data indicate that RANKL-stimulated DC are functionally more efficient tolerogenic APC than unstimulated DC, and promote a greater level of oral tolerance than do their unstimulated counterparts. It is unlikely that the enhanced tolerance observed in RANKL-treated mice may be attributed to the previously described effect of RANKL as a DC survival factor (17, 29), since RANKL treatment did not appear to alter the survival of mucosal DC in vitro. DC are ostensibly the most potent APC and are described as professional APC, being equipped with all the necessary apparatus to promote active immune responses (1). Recently, we and others have expounded on the functionality of this cell type by demonstrating their ability to also behave as tolerogenic APC (3–5). An important issue that such studies have raised is the question of how can one cell type be concerned with the induction of both tolerance and active immunity, and when and how do DC decide to be tolerogenic vs immunogenic? We hypothesize that the location of the DC population may be an important determining factor. The intestine is constantly being challenged by both innocuous Ag, such as essential dietary proteins, and potentially harmful pathogens, such as Salmonella, Yersinia, and Shigella species. It is critical that the cellular components of the intestinal immune system be able to distinguish between these two different types of encounter and not mount inappropriate inflammatory responses to every antigenic challenge. We have previously shown that intestinal DC are specialized to carry out this dual functionality by employing tolerance as their default response to oral Ag, but that they can be converted into immunogenic APC upon activation with proinflammatory cytokines such as IL-1 (10) or, as others have demonstrated, when there is ongoing local inflammation (31, 32). The functional duality of intestinal APC and the regulatory processes that control it are paramount for maintaining health. The fact that transient food/cow’s milk protein intolerance is a common consequence of gastroenteritis is testament to the importance of intestinal immunoregulation. This report clearly shows that mucosal DC may be functionally distinct from peripheral DC, a finding with important biological implications for mucosal vaccine development. This study serves to highlight the differences between mucosal DC and peripheral DC and draws attention to the putative role of DC and expression of the DC molecule, RANK, in tolerogenic Ag presentation following soluble Ag feeding.

References


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