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Cross-Linking of the Mannose Receptor on Monocyte-Derived Dendritic Cells Activates an Anti-Inflammatory Immunosuppressive Program

Marcello Chieppa,* Giancarlo Bianchi,† Andrea Doni,* Annalisa Del Prete,* Marina Sironi,* Gordana Laskarin,‡ Paolo Monti,† Lorenzo Piemonti,† Andrea Biondi,‡ Alberto Mantovani,*§ Martino Introna,* and Paola Allavena‡*§

Immature monocyte-derived dendritic cells (DC) strongly express the endocytic mannose receptor (MR). Addition of a specific anti-MR mAb (clone PAM-1) for 24 h to cultures of immature DC induced phenotypical and functional maturation of the cells, assessed as up-regulation of costimulatory molecules and CD83, and chemotactic response to CCL19. A different isotype-matched anti-MR mAb (clone 19.2) had no significant effect. Engagement of MR with mAb PAM-1 induced the production of the anti-inflammatory cytokines IL-10, IL-1Ra antagonist, and of the nonsignaling IL-1R type II. In contrast, IL-1β, TNF, and IL-12 were not produced. PAM-1-treated DC were unable to polarize Th1 effector cells and did not secrete the chemokines CXCL10 and CCL19; in turn, they produced large amounts of CCL22 and CCL17, thus favoring the amplification of Th2 circuits. T cells cocultured with PAM-1-matured DC initially proliferated but later became anergic and behaved as suppressor/regulatory cells. Natural ligands binding to MR had differential effects. MUC III (a partially purified mucin), biglycan (a purified complex proteoglycan), and mannosylated lipopolysaccharide from Mycobacterium tuberculosis affected cytokine production with high IL-10, IL-1Ra antagonist, IL-1R type II, and inhibition of IL-12. In contrast, mannan, dextran, and thyroglobulin had no significant effect. In conclusion, the appropriate engagement of the MR by mAb PAM-1 and selected natural ligands elicit a secretory program in mono-derived DC characterized by a distinct profile of cytokines/chemokines with the ability to dampen inflammation and to inhibit the generation of Th1-polarized immune responses. The Journal of Immunology, 2003, 171: 4552–4560.

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away from the MHC class II compartments (18). Although structurally related, DEC205 and MR show several distinct characteristics and have a different cell distribution and regulation. DEC205 is absent from macrophages and is highly up-regulated in DC upon maturation, while MR is down-regulated by inflammatory cytokines and up-regulated by Th2-derived cytokines, including IL-4, IL-13, and IL-10 (2–4, 19).

Other evidence points to a role of the MR in adaptive immunity. A construct containing the cysteine-rich domain of MR was found to bind ligands expressed on marginal metallophilic macrophages of the spleen, follicular DC, and subcapsular macrophages of lymph nodes, suggesting that MR may transport Ags to sites where humoral responses occur (20).

The CRDs of the MR, in addition to pathogen molecules, bind also endogenous ligands, including neutrophil-derived myeloperoxidase, tissue plasminogen activator, and lysosomal hydrolases (3, 14). This has suggested that MR might be an important player in the clearance of reactive substances to maintain homeostasis. This consideration has been recently confirmed in vivo in MR−/− mice that have elevated levels of several glycoproteins associated with inflammation (21).

Only few studies have highlighted that MR and other members of the C-type lectin family may participate in signal transduction events, leading either to cell activation (22–24) or inhibition through interference with Toll-like receptor-mediated signaling (25–28).

We previously characterized an anti-MR mAb (clone PAM-1) which strongly stains immature monocyte-derived DC (9, 19, 29). In the present study, we examined whether the cross-linking of MR with this specific mAb had functional effects on DC in vitro. We found that addition of mAb PAM-1 to cultures of immature DC activates a maturation program. Despite expression of a mature phenotype, these cells secreted high levels of several inflammatory factors: IL-1R antagonist (IL-1ra) and IL-1R type II (IL-1RII), which antagonize the effects of IL-1 (30–32) and IL-10. PAM-1–treated DC did not produce IL-12, were unable to polarize Th1 effectors, and promoted Th cell anergy. These DC also produced the chemokines CCL17 and CCL22 that would eventually amplify Th2 and Treg cells and negatively modulate Th1 polarization. These findings indicate that cross-linking of the endocytic MR on DC may provide a novel tool to analyze the functional plasticity of DC and to tune inflammation and adaptive immunity.

Materials and Methods

Reagents

Human recombinant GM-CSF and IL-4 were donated from Schering-Plough Research Institute (Dardilly, France); IL-13 was a gift from Sanofi Elf Bio Recherches (LaBègue, France); and LPS (Escherichia coli 055:BS) was purchased from Sigma-Aldrich (St. Louis, MO). CCL3 and CCL19 were obtained from PeproTech (Rocky Hill, NJ). Natural ligands of MR were all purchased from Sigma-Aldrich and included FITC-dextran, MUC protein G columns. mAb PAM-1 immunoprecipitated a protein of 180 kDa from surface biotinylated immature DC as described previously (9). F(ab′)2 of mAb PAM-1 were prepared using a commercial kit (Pierce, Rockford, IL) following the manufacturer’s instructions. Chinese hamster ovary (CHO) parental cell line and CHO cells transfected with the human MR (CHO-MR) were a kind gift from Prof. L. S. Schlesinger (University of Iowa, Iowa City, IA) (34, 35).

Preparation and activation of DC cultures

Blood samples were collected at the Transfusion Center of General Hospital (Desio, Milan, Italy) under protocols approved by the board of the local ethical Committee. DC were differentiated in vitro as previously described (36–38). Blood monocytes were purified by Ficoll and Percoll gradients, followed by plastic adherence, and were cultured for 6 days at 1 × 10⁶/ml in medium RPMI 1640 (Biochrom, Berlin, Germany) and 10% FCS (HyClone, Logan, UT) with 50 ng/ml GM-CSF and 20 ng/ml IL-13. In some experiments, DC were prepared with GM-CSF and IL-4 (20 ng/ml), with identical results as far as differentiation and functional activity. After 6 days of culture, the outcome population consisted of typical immature DC which usually expressed CD1a (>90% positive cells), low levels of CD80 and CD86, and was negative for CD83 (<10%) and CD14 (<10%) (36–38). To induce terminal maturation, LPS (10 ng/ml), PAM-1 (2 μg/ml) F(ab′)2-PAM-1 (10 μg/ml), or mAb 19.2 (2 μg/ml) were added at day 6 for 24 h. PAM-1 preparations were checked throughout the study and found to be endotoxin free by Limulus assay (BioWhittaker).

FACS analysis

Cell staining was performed using PE-conjugated mouse mAbs anti-CD1a, CD86, CD83, and CD80 (BD PharMingen). Negative controls were stained with isotype-matched irrelevant mAb followed by PE-goat anti-mouse IgG (BD PharMingen). Cells were analyzed on a FACSCalibur by collecting 10,000 cells/sample, gated based on forward and side scatter. Results are expressed as percentage of positive cells or as mean fluorescence intensity (MFI) under logaritmonic scale. MFI were calculated as follow: MFI of sample cells – MFI of negative control.

Mixed leukocyte reaction

Irradiated control or treated DC were added in graded doses to 1 × 10⁶ purified allogeneic T cells from cord blood in 96-well round-bottom micro-culture plates. Each group was performed in triplicates. [3H]Thymidine incorporation was measured on day 5 by a 16-h pulse (5 Ci/μl; Amer sham, Buckingham, U.K.).

Migration assay

Cell migration was evaluated using a chemotaxis chamber (Neuroprobe, Pleasanton, CA) and polycarbonate filters (5-μm pore size; Neuroprobe) as previously described (39). Results are expressed as the net number of migrated cells (in five high-power fields) over medium (absence of chemokine in lower wells). Each experiment was performed in triplicates.

Cytokine and chemokine production

Control or treated DC were plated at 10⁶/ml and incubated for 24 h with the designed treatments. Cell-free supernatants were harvested and tested in sandwich ELISA, CCL22, CCL2, and IL-1RII were quantified as described elsewhere (30, 40, 41). IL-1, IL-1ra, IL-10, IL-12p70, TNF α, CXCL8, CCL19, CCL17, and CXCL10 were determined by ELISA purchased by R&D Systems (Minneapolis, MN).

Polarization of naive T lymphocytes

Cord blood from normal end-stage deliveries was obtained from the Department of Obstetrics and Gynecology (University of Milan, Milan, Italy) under protocols approved by the board of the local ethical Committee. Naive T cells were cultured on CD69-coated plastic dishes and cocultured (1 × 10⁶/ml) for 6 days with irradiated untreated (immature) or treated DC at a 10:1 ratio. At the end of the incubation, cells were collected and stimulated with PMA and ionomicin (Sigma-Aldrich) for 6 h in the presence of 10 μg/ml brefeldin for the last 2 h. Cells were fixed and stained with FITC-anti-IFN-γ and PE-anti-IL-4 mAb (BD PharMingen).

T cell anergy assay

Irradiated control or treated DC were cocultured with allogeneic T cells at a DC:T ratio of 1:10 for 6 days. T cells were then harvested, extensively washed, and rested in medium without exogenous stimuli. After 2 days, T cells were cultured again with fully mature LPS-DC from the same donor as the first priming or DC from an unrelated donor. [3H]Thymidine incorporation was measured after 3 days of culture.
a 10:1 ratio. T cells were then washed and rested in medium alone for 2 days. T cells were then mixed with freshly isolated T cells at ratios ranging from 1:1 to 4:1 (responder:suppressor ratio) and with allogenic DC (10:1). [3H]Thymidine incorporation was measured after 5 days of culture.

Statistical analysis
The Mann-Whitney U test was used to compare differences in cytokine production.

Results
mAb PAM-1 recognizes a functional epitope of the human MR
We previously reported that mAb PAM-1 immunoprecipitates a protein with a Mr of 180 kDa, as expected for the MR and strongly stains in vitro immature monocyte-derived DC and in vivo sinusaline macrophages of spleen and lymph nodes (9, 19, 29). The specificity of PAM-1 for the MR was confirmed in this study by staining of MR-transfected CHO cells (Fig. 1A). Although the staining profile of mAb PAM-1 on immature DC was almost identical to that of a commercially available anti-MR mAb (clone mAb 19.2; Fig. 1B), the functional activity of these two mAbs was markedly different, as mAb PAM-1 reduced by $>75\%$ the internalization of FITC-dextran, a ligand for MR, while mAb 19.2 was a poor competitor (Fig. 1, C and D).

Cross-linking of MR with mAb PAM-1 induces a maturation program in DC
To study the effect of MR engagement by mAb PAM-1 on immature DC, purified mAb PAM-1 (2 μg/ml) was added to cultures of monocyte-derived DC at day 6 for 24 h and a phenotype analysis was performed. The expression of CD80, CD83, and CD86 was strongly increased in DC treated with mAb PAM-1, with levels similar to those obtained by exposure to an optimal concentration of LPS (10 ng/ml). Fig. 2A shows a representative experiment. In a series of five experiments, MFI for CD83 was $35 \pm 12$ (mean $\pm$ SE).
PAM-1 and LPS-treated DC, respectively (n/H11006 MFI increased from 58 matured and LPS-matured DC, respectively (n/H11005). 2 of PAM-1 were able to trigger the detectable endotoxin. F(ab’)2 contamination by PAM-1 was carefully tested throughout the study for endotoxin. MR 19.2 mAb (2/H9262) and was also ineffective at higher concentrations (10/B2). B2 phenotype, even after cross-linking with goat anti-mouse Ig (Fig. 2/H9262). Matched (IgG1) anti-MR mAb 19.2 had no significant effect on DC activation through MR, although less potently than the endotoxin. Anti-MR PAM-1 did not prevent the maturation effect induced by mAb PAM-1 (Fig. 2/B2). Puriﬁed mAb PAM-1 was carefully tested throughout the study for endotoxin contamination by Limulus assay and always found to be free of detectable endotoxin. F(ab’)2 of PAM-1 were able to trigger the activation of DC through MR, although less potently than the entire mAb. At 10/μg/ml, F(ab’)2-PAM-1 were as active as the entire mAb (Fig. 2/B2), but lower doses (2 and 5/μg/ml) were ineffective or partially effective (data not shown). In contrast, the isotype-matched (IgG1) anti-MR mAb 19.2 had no significant effect on DC phenotype, even after cross-linking with goat anti-mouse Ig (Fig. 2/B2) and was also ineffective at higher concentrations (10/μg/ml; data not shown). Interestingly, pretreatment of DC with mAb anti-MR 19.2 did not prevent the maturation effect induced by mAb PAM-1 (Fig. 2/B2), suggesting that the two Abs recognize different epitopes of the MR.

We next examined the migratory activity in response to chemokines of DC treated with PAM-1. A hallmark of mature DC is the down-regulation of the chemokine receptor CCR5 and the up-regulation of CCR7. This receptor switch is instrumental for the correct positioning of mature DC in secondary lymphoid organs (42–45). In a chemotaxis assay, as expected, immature DC migrated to CCL3 and poorly to CCL19. PAM-1-treated DC, similarly to LPS-matured DC, lost the capacity to migrate in response to CCL3 and acquired responsiveness to CCL19 (Fig. 3). Therefore, treatment with mAb PAM-1 induced a correct chemokine receptor switch.

Cross-linking of MR with mAb PAM-1 induces the secretion of anti-inﬂammatory cytokines and Th2-attracting chemokines

The secretory activity of DC is of major importance to initiate, amplify, and orientate the immune response. Cytokine and chemokine production was measured in supernatants of DC treated for 24 h with PAM-1. Unlike LPS-matured DC, DC treated with PAM-1 alone were unable to produce IL-12, TNF, and IL-1 (Fig. 4). In contrast, they produced substantial levels of the immunosuppressive cytokine IL-10, IL-1ra, and IL-1RII, two molecules which antagonize IL-1 activity (30–32, 46). Treatment of DC with anti-MR clone 19.2 was always ineffective (Fig. 4). DC treated with F(ab’)2-PAM-1 were tested for the production of IL-1RII. Of three experiments performed, mean ± SE values of IL-1RII were 2.6 ± 0.3, 2.3 ± 0.5 and 0.2 ± 0.04 ng/ml for DC treated with PAM-1 and DC treated with F(ab’)2-PAM-1 and immature untreated DC, respectively (data not shown).

Engagement of the MR by PAM-1 mAb induced the production of large quantities of the chemokines CCL22 and CCL17 (Fig. 5). CXCL8 and CCL2 were also produced, but to a lower extent (Fig. 5). CCL17 and CCL22 are two chemokines attracting Th2 effectors and regulatory T cells (47–50). In contrast to LPS treatment, DC treated with mAb PAM-1 expressed very low or undetectable amounts of CXCL10 and CCL19 (Fig. 5). CXCL10 is a ligand of CXCR3, a receptor preferentially expressed on Th1 cells, while CCL19, which interacts with CCR7, plays a well-established role in the recruitment of DC precursors and T cells in secondary lymphoid organs (47–49, 51). Thus, treatment of monocyte-derived DC with mAb PAM-1 alone induces the production of a distinct set of chemokines that would eventually favor the recruitment of Th2 and Treg cells and negatively regulate a Th1-type immune response. Anti-MR 19.2 was tested in some experiments and never modiﬁed chemokine production of DC (data not shown).

FIGURE 3. Chemotactic activity of PAM-1-matured DC. Chemotactic activity in response to CCL3 (100 ng/ml) and CCL19 (100 ng/ml) of differently treated DC. LPS (10 ng/ml) and anti-MR PAM-1 (2/μg/ml) were added to immature DC (day 6) for 24 h. Results are net numbers (mean ± SE of five microscope ﬁelds) of migrated cells over medium (absence of chemokine in lower wells). Data are representative of three experiments. *, Signiﬁcantly different (p < 0.01) compared with untreated cells (immature DC). Ctrl, Control.

FIGURE 4. Cytokine production by PAM-1-treated DC. A, Immature DC were treated for 24 h with LPS (10 ng/ml), anti-MR PAM-1 (2/μg/ml), or anti-MR 19.2 mAb (2/μg/ml). Cell supernatants were tested in ELISA for IL-10, IL-12p70, TNF, IL-1, IL-1ra, and IL-1RII. Shown are representative experiments of two to seven performed. #, Signiﬁcantly different (p < 0.01) compared with untreated cells (Im.DC). NT, Not tested.
PAM-1-matured DC induce T cell anergy

We next examined the accessory cell activity of PAM-1-treated DC in classical MLR assays. DC treated for 24 h with mAb PAM-1 initially triggered the proliferation of allogenic naive T cells and were better accessory cells compared with immature DC (Fig. 6A). These APC, however, were unable to sustain T cell proliferation over time. Fig. 6B shows that T cells that were first primed with PAM-1-matured DC were poor responders when rechallenged with fully mature LPS-DC from the same donor. Stimulation with LPS-DC from a different donor or addition of IL-2 did not overcome this anergic state (Fig. 6B). In contrast, T cells first primed with mature LPS-DC had the potential to proliferate when rechallenged 1 wk later with the DC from the same donor (Fig. 6B). Therefore, T cells activated by PAM-1-treated DC become unresponsive to a second challenge in an alloantigen-nonrestricted manner. Moreover, these T cells behaved as suppressor/regulatory cells when mixed with other T cells in an independent MLR assay (Fig. 6C). At 1:1 ratio (T cell responder:suppressor T cells), T cells primed with PAM-1-DC inhibited by 70% the proliferation of responder T cells and at 2:1 ratio gave 30% inhibition, while T cells primed by LPS-DC were not inhibitory.

We next tested the cytokine-producing ability of T cells cocultured with PAM-1-DC. Fig. 6D shows a representative experiment. While LPS-matured DC polarized naive lymphocytes mainly into IFN-γ-producing cells, PAM-1-treated DC were unable to polarize Th1 effectors. In a series of four experiments, mean values of IFN-γ-producing cells was 55 ± 8%, 16 ± 9%, and 12 ± 10% for LPS-treated, PAM-1-treated, and untreated immature DC, respectively. A slight increase in the proportion of IL-4-producing cells was observed with PAM-1-matured DC compared with immature DC (9 ± 2% and 6 ± 2%, respectively).

Cross-linking of MR with mAb PAM-1 induces a high IL-10, low IL-12 cytokine profile

We next concentrated our efforts on the LPS-induced production of IL-10 and IL-12, as the balance between these two cytokines is of major importance in the APC activity and Th-polarizing function of DC. Addition of mAb PAM-1 to LPS-treated cells significantly increased IL-10 and inhibited IL-12 secretion (Fig. 7). In a series of seven experiments, IL-10 production was 2.3 ± 0.3 and 5.7 ± 0.8 ng/ml (mean ± SE) from LPS-treated and LPS plus PAM-1-treated DC, respectively (p 0.01). F(ab')2-PAM-1 also increased IL-10 release (4.8 ± 0.9, p < 0.05, n = 3; Fig. 7). IL-12 production was 4.3 ± 0.9 ng/ml in LPS-treated DC and 2.0 ± 0.7

FIGURE 5. Chemokine production by PAM-1-treated DC. Immature DC (Im.DC) were treated with LPS (10 ng/ml) or anti-MR PAM-1 (2 μg/ml) for 24 h. Cell-free supernatants were collected and tested in ELISA for CCL17, CCL22, CXCL8, CCL2, CXCL10, and CCL19. Shown are representative experiments of two to five performed. * Significantly different (p < 0.01) compared with untreated cells (immature DC).

FIGURE 6. PAM-1-DC induce T cell anergy and do not polarize Th1 effectors. A, Proliferative response of allogenic naive T cells cocultured for 6 days with DC treated with LPS (10 ng/ml) or PAM-1 (2 μg/ml). B, Proliferative response of cultured T cells primed as specified in the legend and then restimulated with LPS-DC from the same donor (continuous line) or from an unrelated donor (dotted line). Selected samples received 100 U/ml IL-2. Representative of two experiments. C, Suppressor cell activity of T cells originally primed by PAM-1-DC or LPS-DC. T cells were mixed at the indicated ratio with freshly isolated T cells and LPS-DC (10:1). Shown is a representative experiment of three performed. D, Polarization of naive T cells. Intrastaining cytokine production by T cells cocultured for 7 days with differently treated DC (10:1). T cells were stained with FITC-anti-IFN-γ and PE-anti-IL-4 and analyzed by flow cytometry. Data are representative of four experiments.
FIGURE 7. IL-12 and IL-10 production by DC treated with LPS plus PAM-1. Immature DC were treated for 24 h with LPS (10 ng/ml) or a combination of LPS and anti-MR mAbs: PAM-1 (2 μg/ml), F(ab')2-PAM-1 (10 μg/ml), and 19.2 mAb (2 μg/ml). Selected cultures received anti-IL-10 (2 μg/ml). Upper panels, Production of IL-10 and IL-12p70 in ELISA. Results are the mean ± SE of seven experiments for cells with LPS + PAM-1 and three experiments with LPS plus F(ab')2-PAM-1. * p < 0.05; ** p < 0.01 compared with LPS-DC. # p < 0.01 compared with LPS plus PAM-1-DC. Lower panels, Kinetics of IL-10 and IL-12p70 production by DC treated with LPS or LPS plus PAM-1. CTRL, Control.

ng/ml in DC treated with LPS plus PAM-1 (p < 0.01, n = 7) and 2.3 ± 0.8 with cells treated with F(ab')2-PAM-1 (p < 0.05, n = 3; Fig. 7). Addition of anti-MR mAb 19.2 to LPS-treated cells, at doses of 2 μg/ml, did not significantly modify the secretion of IL-10 or IL-12. To verify whether the increased amounts of endogenous IL-10 could negatively affect IL-12 production, cells were treated with anti-IL-10 mAbs and stimulated with the combination of mAb PAM-1 and LPS. Under these conditions, neutralization of IL-10 resulted in higher production of IL-12 to levels comparable to those obtained with LPS alone (Fig. 7).

Fig. 7 (lower panels) shows the kinetics of IL-10 and IL-12 production in LPS-DC treated with mAb PAM-1. IL-10 release was detectable after 6 h and progressively increased; IL-12 production was already inhibited after 12 h compared with LPS-treated cells. A similar increase in IL-10 production and decrease in IL-12 by DC treated with mAb PAM-1 was observed also when the cells were matured with CD40 ligand (data not shown). Overall, these results demonstrate that after cross-linking of the MR with mAb PAM-1, the cytokine production induced by maturation stimuli in DC is shifted to an increased production of IL-10 and concomitant decrease of IL-12.

Selected natural ligands of MR affect cytokine production of DC

It was important to verify whether engagement of MR with natural ligands could mimic the effects mediated by mAb PAM-1. The mannosylated lipooligosaccharide (ManLAM) from M. tuberculosis is a well-known ligand of MR (3, 14, 25, 52). We also tested a partially purified mucin extracted from the gastrointestinal tract, named MUC III, and a purified complex proteoglycan, biglycan, consisting of a core protein and two glycosaminoglycans, extracted from cartilage. These ligands were first tested for their ability to bind to MR on immature DC in a competition assay with FITC-dextran uptake. DC pretreatment with MUC III, biglycan, and ManLAM significantly and dose-dependently reduced the internalization of FITC-dextran (Fig. 8A).

The cytokine production of DC treated with MR ligands is shown in Fig. 8, B and C. As observed with mAb PAM-1, treatment with MUC III, ManLAM, and biglycan significantly increased IL-10 production and decreased IL-12 in LPS-maturing DC (Fig. 8B). Secretion of IL-10 in the absence of LPS was occasionally observed in DC treated with MUC III and ManLAM, with donor variability (data not shown). Treatment of immature DC with biglycan, MUC III, and ManLAM also induced a significant production of IL-1ra and IL-1RII, but not of IL-1 (Fig. 8C). The carbohydrates mannan and dextran and the glycoprotein thyroglobulin were also studied and had no significant effect on cytokine secretion (data not shown).

These results indicate that some ligands of the MR indeed have the ability to activate DC in an anti-inflammatory mode, as observed with the mAb-mediated cross-linking of the receptor. The reason why some MR ligands functionally activate MR and others do not is at the moment unclear. It may be that only ligands binding to specific domain(s) fully engage the receptor or that only ligands with peculiar structures are effective. The latter hypothesis is supported by the recent finding that aggregates of ManLAM have a higher affinity for MR (53).

Discussion

In this study, we show that cross-linking of the MR with the specific mAb PAM-1 activates a maturation program in monocyte-derived DC characterized by up-regulation of CD83 and costimulatory molecules and migration in vitro in response to the chemokine CCL19. PAM-1 matured-DC, however, showed a distinct cytokine and chemokine repertoire compared with classically activated LPS-DC: they were unable to produce IL-12 and mAb PAM-1 significantly inhibited IL-12 secretion by LPS-stimulated...
cells. On the other hand, these DC produced substantial levels of IL-10, and the combined stimulation of LPS and PAM-1 resulted in additive or more than an additive response in terms of IL-10 release. Of note, DC treated with mAb PAM-1 secreted high levels of two anti-inflammatory mediators, IL-1ra and IL-1RII, which antagonize the effects of IL-1 in vitro (30, 31) and also in vivo (32).

The ability of DC to secrete chemokines is of great importance to amplify and orientate the type of immune response. PAM-1-treated DC secreted copious amounts of the CCR4 ligands CCL17 and CCL22. CCR4 is expressed and functional in polarized Th2 and T regulatory cells (47–50). Unlike DC stimulated with LPS, they were unable to produce CXCL10, a ligand for CXCR3, expressed in Th1 and NK cells (47, 48), and CCL19 a ligand for CCR7. The chemokine CCL19 modulates the migration of naive T cells, which will negatively regulate Th1-polarized responses. Thus, the nature and structure of the ligand may determine the fate of anti-inflammatory and tolerogenic cytokines, which would prevent the generation of Th1-polarized responses. This contrasts with previous studies showing that C-type lectins could enhance Ag presentation to T cells (15–17). More recent studies, however, have demonstrated that Ag targeting to DEC205 receptors also induced a state of T cell anergy after an initial stimulation (18). These authors proposed that the targeting of DEC205 in steady-state conditions (i.e., in the absence of inflammatory/immunological stimuli) would result in peripheral tolerance (54, 55). Our study suggests that mediators of this tolerance are the anti-inflammatory cytokines and Th2-recruiting chemokines produced after MR cross-linking.

Endocytic receptors represent a port of entry of several pathogens. Emerging evidence indicates that C-type lectin receptors may be exploited by pathogens and tumor Ags to escape immune reaction. In fact, carbohydrates binding to C-type lectin receptors may interfere with the internalization routing of an Ag. Carbohydrates with multiple repeats may engage multiple ligands, resulting in a supercross-linking of the receptors and block or engulfment of the endocytosis process. Retention in early endosomes would prevent the subsequent routing to late endosome and lysosomes and therefore inhibit Ag processing and presentation. The supercross-linking of C-type lectin receptors by multiple ligands has been shown with the tumoral mucin MUC1, which expresses multiple tandem repeats, and also by pathogen’s carbohydrates, including ManLAM. Nigou et al. (53) recently reported that only multiacylated ManLAM forms aggregates that have higher affinity for the MR. Thus, the nature and structure of the ligand may determine treated DC eventually became hyporesponsive and behaved as suppressor/regulatory cells. Thus, engagement of the MR with mAb PAM-1 generates APC-promoting T cell anergy.

The mAb PAM-1 recognizes the human MR as shown previously (9, 19, 29) and confirmed in this study with MR-transfected cells. The observed effect on DC activation was specific to PAM-1 as a different anti-MR mAb (clone 19.2) of the same isotype did not induce maturation or cytokine modulation. Other isotype-matched mAbs reacting with unrelated surface molecules expressed on DC were similarly inactive (data not shown). The functional effects activated by mAb PAM-1 are not induced via its Fc fragment; in fact, F(ab’)_2 of PAM-1 were sufficient to up-regulate the expression of a mature phenotype and cytokine production. Moreover, saturation of FcRs on DC with the inactive mAb 19.2 or with human Ig did not prevent the activation effect of PAM-1. These results also indicate that the two mAbs, PAM-1 and 19.2, recognize different epitopes of the MR. This is supported also by the finding that mAb 19.2 shows lower efficiency in competition assay with FITC-dextran for MR binding.

To verify whether these functional effects observed after engagement of MR were unique of treatment with mAb PAM-1, we tested several natural MR ligands. Among them, MUC III, a partially purified mucin from the gastrointestinal tract and biglycan, a complex proteoglycan of the extracellular matrix, significantly modulated cytokine secretion in terms of high anti-inflammatory cytokines and inhibition of IL-12 production. A pathogen-derived MR ligand, ManLAM, from M. tuberculosis, was also tested. It was already reported that ManLAM inhibits IL-12 production by LPS-treated DC (25). In this study, we confirmed this finding and further extended it to show that ManLAM induced the production of high levels of IL-1ra, IL-1RII, and IL-10. Therefore, other MR ligands of natural origin produced similar effects as those observed with mAb PAM-1 treatment.

The concept emerging from this study is that MR engagement on myeloid DC, with selected ligands or Abs, activates an alternative maturation program characterized by a profile of anti-inflammatory and tolerogenic cytokines, which would prevent the generation of Th1-polarized responses. This contrasts with previous studies showing that C-type lectins could enhance Ag presentation to T cells (15–17). More recent studies, however, have demonstrated that Ag targeting to DEC205 receptors also induced a state of T cell anergy after an initial stimulation (18). These authors proposed that the targeting of DEC205 in steady-state conditions (i.e., in the absence of inflammatory/immunological stimuli) would result in peripheral tolerance (54, 55). Our study suggests that mediators of this tolerance are the anti-inflammatory cytokines and Th2-recruiting chemokines produced after MR cross-linking.
how the receptor is engaged and multiple engagement may affect the intracellular routing of the ligand. This would in part explain why we found that some ligands of MR have functional effects on the cells and others have not. Anti-MR Abs are likely to simultaneously engage many receptors with high affinity.

Another mechanisms to escape the immune system is to interfere with the signaling of C-type lectins. It has been reported that ManLAM inhibits IL-12 production induced by activation through Toll-like receptors (25) and that cross-linking of BDCA-2, a type II C-type lectin, strongly down-regulates the production of IFN-γ induced by a variety of stimuli (26). Thus, C-type lectins can deliver negative signals to the cells. Although some components of the man-binding C-type lectin superfamily have signaling motifs in their cytoplasmic domains, MR and also BDCA-2 have not, and this raises the intriguing question as to whether these receptors associate to adapter molecules to transduce their signals.

Experiments aimed at identifying the signaling pathway triggered after cross-linking of the MR were undertaken during this study. Electromobility shift assay with nuclear extracts from PAM-1-treated DC revealed no transcriptional activation of NF-κB nor degradation of the IkB protein (data not shown); in contrast, pretreatment of cells with wortmannin (phosphatidylinositol 3-kinase inhibitor) significantly inhibited the increase of CD83 expression and the release IL-1RII and IL-10 induced by mAb PAM-1 (data not shown). These preliminary experiments suggest that phosphatidylinositol 3-kinase appears to be involved in the signaling downstream MR cross-linking.

A role for MR in maintaining homeostasis was recently demonstrated in mice genetically deficient for MR (21). MR−/− mice have elevated levels of lysosomal hydrolys and several other glycoproteins which are up-regulated during inflammation and tissue remodeling. Of note, mediators that are all associated with the resolution of inflammation, including IL-4, IL-13, and PGE, increase MR expression and enhance the removal of lysosomal enzymes (3). It is of interest that MR expression is higher in interstitial DC of mucosal surfaces (lung, gut) that produce IL-10 and/or TGFβ and are considered tolerogenic APC (56, 57). In contrast, DC from epidermis, T cell areas of lymph nodes, and spleen marginal zone, which are considered immunostimulatory class II-positive lysosomal compartments (58), do not express MR (8, 58).

In conclusion, in line with the hypothesis that MR is an important regulator of homeostasis, this study has demonstrated that engagement of the MR activates the secretion of cytokines and chemokines (IL-10, IL-1α, IL-1RII, CCL17, CCL22) that contribute to control inflammation and to down-regulate Th1-polarized immune responses. The functional plasticity of DC after cross-linking of the MR may be exploited to tune inflammation and adaptive immunity.

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References
