NIH Public Access
Author Manuscript
J Immunol. Author manuscript; available in PMC 2009 March 4.

Published in final edited form as:
J Immunol. 2007 December 15; 179(12): 8128–8136.

LILRA2 Activation Inhibits Dendritic Cell Differentiation and Antigen Presentation to T Cells[1]

Delphine J. Lee[*], **Peter A. Sieling**[*], **Maria Teresa Ochoa**[*], **Stephan R. Krutzik**[*], **Beichu Guo**[†], **Maristela Hernandez**[¶], **Thomas H. Rea**[§], **Genhong Cheng**[†,‡], **Marco Colonna**[¶], and **Robert L. Modlin**[*,†,2]

* *Division of Dermatology, Department of Medicine, David Geffen School of Medicine, University of California, Los Angeles, CA 90095*

† *Department of Microbiology, Immunology and Molecular Genetics, University of California, Los Angeles, CA 90095*

‡ *Jonsson Comprehensive Cancer Center, University of California, Los Angeles, CA 90095*

§ *Department of Dermatology, University of Southern California School of Medicine, Los Angeles, CA 90033*

¶ *Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO 63110*

Abstract

The differentiation of monocytes into dendritic cells (DC) is a key mechanism by which the innate immune system instructs the adaptive T cell response. In this study, we investigated whether leukocyte Ig-like receptor A2 (LILRA2) regulates DC differentiation by using leprosy as a model. LILRA2 protein expression was increased in the lesions of the progressive, lepromatous form vs the self-limited, tuberculoid form of leprosy. Double immunolabeling revealed LILRA2 expression on $CD14^+$, $CD68^+$ monocytes/macrophages. Activation of LILRA2 on peripheral blood monocytes impaired GM-CSF induced differentiation into immature DC, as evidenced by reduced expression of DC markers (MHC class II, CD1b, CD40, and CD206), but not macrophage markers (CD209 and CD14). Furthermore, LILRA2 activation abrogated Ag presentation to both CD1b- and MHC class II-restricted, *Mycobacterium leprae*-reactive T cells derived from leprosy patients, while cytokine profiles of LILRA2-activated monocytes demonstrated an increase in TNF-α, IL-6, IL-8, IL-12, and IL-10, but little effect on TGF-β. Therefore, LILRA2 activation, by altering GM-CSF-induced monocyte differentiation into immature DC, provides a mechanism for down-regulating the ability of the innate immune system to activate the adaptive T cell response while promoting an inflammatory response.

The ability of the innate immune system to activate the adaptive T cell response is part of an effective host defense against intracellular pathogens. This role of the innate immune system is primarily mediated by dendritic cells (DC),[3] professional APCs (1) that are highly efficient in activation of T cell responses that provide cell-mediated immunity against the pathogen

[1]This work was supported by National Institutes of Health Grants AR40312 and AI22553 (to R.L.M.), AR053104-01 (to D.J.L.), and AI056154 and AI069120 (to G.C.); the Dermatology Foundation (to D.J.L.); and the Lymphoma and Leukemia Society (to B.G.).

2Address correspondence and reprint requests to Dr. Robert L. Modlin, University of California-Los Angeles, Division of Dermatology, 10833 LeConte Avenue, 52-121 Center for the Health Sciences, Los Angeles, CA 90095. E-mail address: rmodlin@mednet.ucla.edu.

Disclosures

The authors have no financial conflict of interest.

[3]Abbreviations used in this paper: DC, dendritic cell; iDC, immature dendritic cell, L-lep, lepromatous leprosy; T-lep, tuberculoid leprosy; LILR, leukocyte immunoglobulin-like receptor; *M.*, *Mycobacterium.*

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

(2). At the site of infection, inflammatory cytokines trigger monocytes to differentiate into immature DC (iDCs), providing one mechanism by which the innate immune response triggers the adaptive T cell response (3).

Ag-specific T cell responses are required for effective host defense in human leprosy, a disease caused by infection with the intracellular bacterium *Mycobacterium. leprae* (4,5). The disease forms a spectrum in which the clinical responses correlate with the level of the immune response to the pathogen (6). At one pole of the spectrum, patients with tuberculoid leprosy (T-lep) manifest a localized form of the disease with few bacilli present and strong cell-mediated immunity to *M. leprae*. At the opposite pole, patients with lepromatous leprosy (L-lep) suffer from a more disseminated form of the infection with numerous bacilli within macrophages and lack effective cell-mediated immunity to the pathogen. The clinical spectrum of leprosy provides an opportunity to assess immunoregulatory mechanisms of innate and adaptive immunity that contribute to the outcome of the infection.

From the study of leprosy in humans, we have gained insight into the immunoregulatory role of leukocyte Ig-like receptor (LILR) family (7–9) of genes (10). LILRs are expressed on lymphocytes and myelomonocytic cells, including macrophages, mast cells, and dendritic cells (11–13) and are known to regulate both innate and adaptive immune responses (14). Expression of the mRNA encoding several LILR family members, in particular LILRA2, was significantly greater in the lesions of the lepromatous (L-lep) vs the tuberculoid (T-lep) form of leprosy (10). Furthermore, LILRA2 activation increased the IL-10/IL-12 ratio and also inhibited the antimicrobial activity of the innate immune response to mycobacterial TLR2/1 ligands (10). In the present study, using leprosy as a model, we examined whether LILRA2 activation regulates the innate immune system.

Materials and Methods

Patients and clinical specimens

The acquisition of all skin biopsy specimens from leprosy patients and peripheral blood from healthy human donors was reviewed and approved by the committees on investigations involving human subjects of the University of California, Los Angeles. For all procedures, informed consent was obtained. Leprosy patients were recruited on a volunteer basis from the ambulatory population seen at Hansen's Disease Clinic at Los Angeles County/University of Southern California Medical Center. Clinical classification of patients with symptomatic *M. leprae* infection was performed according to the criteria of Ridley and Jopling (6). Patients presenting with de novo tuberculoid leprosy or exhibiting reversal reactions were defined as T-Lep, and those presenting with polar lepromatous were defined as L-lep. Blood samples for isolation of PBMC were obtained by venipuncture from healthy volunteers after obtaining their informed consent. PB-MCs were isolated using Ficoll-Hypaque gradient centrifugation (Ficoll-Paque; Pharmacia Biotech AB).

Ags and Abs

Extracts of *M. tuberculosis* were prepared by probe sonication as previously described (15) and provided by Dr. John Belisle (Colorado State University, Fort Collins, CO). The GroES protein was provided by Dr. Patrick Brennan through a contract with National Institute of Allergy and Infectious Diseases, contract N01-AI-25469, "Leprosy Research Support". The GroES peptide (16) was synthesized by SynPep.

The mouse mAb (IgG2b) specific for human LILRA2 was generated by previously established methods (17). This Ab was used to activate monocytes and for immunohistochemical labeling in leprosy skin lesions. The following mAbs were used for flow cytometry and

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

immunohistochemistry studies: L243 (anti-HLA-DR, BD Biosciences, flow cytometry), BCD1b3.1 (anti-CD1b (18), immunohistochemistry, and flow cytometry), MEM-233 (anti-CD80, Caltag Laboratories, flow cytometry), 2331(FUN-1) (anti-CD86, BD Biosciences, flow cytometry), HB14 (anti-CD40, Caltag Laboratories, flow cytometry), MEM-111 (anti-CD54, Caltag Laboratories, flow cytometry), 1C3 (anti-CD58, BD Biosciences, flow cytometry, GHI/61 (anti-CD163, BD Biosciences, flow cytometry), 19.2 (anti-CD206, BD Biosciences, flow cytometry), CB38 (NL07) (anti-CD36, BD Biosciences, flow cytometry), DCN46 (anti-CD209, BD Biosciences, flow cytometry), TÜK4 (anti-CD14, Caltag Laboratories, flow cytometry), 3G8 (anti-CD16, Caltag Laboratories, flow cytometry), FLI8.26 (anti-CD32, BD Biosciences, flow cytometry), 10.1 (anti-CD64, BD Biosciences, flow cytometry), EBVCS-5 (anti-CD23, BD Biosciences, flow cytometry), M5E2 (anti-CD14, BD Biosciences, immunohistochemistry), PG-M1 (anti-CD68, DakoCytomation, immunohistochemistry), and appropriate isotype controls (Caltag Laboratories, Sigma-Aldrich, and BD Biosciences).

Immunohistochemical studies

Immunoperoxidase-labeling of cryostat sections was performed as described (19). In brief, skin biopsy specimens were embedded in OCT medium (Sakura Finetek) and frozen in a liquid nitrogen-cooled methylbenzene bath. Sections (4 μm thick) were acetone-fixed and blocked with 5% normal horse serum (immunoperoxidase studies) or normal goat serum (immunofluorescence studies) and then incubated with the mAbs for 60 min. Between all incubations, sections were washed twice with PBS for 10 min. For immunoperoxidase studies, biotinylated horse anti-mouse IgG was incubated for 30 min. Primary Ab was visualized with the Vectastain Elite Avidin-biotin complex system (Vector Laboratories), which uses avidin and a biotin-peroxidase conjugate for signal amplification. ABC reagent was incubated for 30 min, washed, then incubated with substrate (3-amino-9-ethylcarbazole) for 10 min. Slides were counterstained with hematoxylin and mounted in crystal mounting medium (Biomeda).

Double immunofluorescence was performed by serial incubation of cryostat tissue sections with mouse anti-human mAbs of different isotypes as described (4). In brief, sections were serially incubated with a mouse mAb against LILRA2 followed by goat anti-mouse IgG2b (Molecular Probes) labeled with fluorochrome (Alexa 568). Sections were washed and incubated with Abs for CD14 (M5E2, BD Biosciences), CD1b (BCD1b3.1 (18) provided by S. A. Porcelli), and CD68 (PG-M1, DakoCytomation) for 1 h followed by incubation with isotype-specific goat anti-mouse IgG Abs (Molecular Probes) labeled with fluorochrome (Alexa 488). Controls included staining with isotype-matched irrelevant Abs. Images were obtained using confocal laser microscopy (University of California, Los Angeles core facility). Immunofluorescence was examined with a Leica TCS SP inverted confocal laser scanning microscope (Leica Microsystems).

Immunoblotting

PBMC were purified by Ficoll-Hypaque gradient centrifugation (Ficoll-Paque; Pharmacia Biotech AB, Uppsala, Sweden). Five million cells were stimulated with anti-LILRA2 Ab or isotype control Ab for various time points. Cells were lysed in lysis buffer (1% TX-100, 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1.0 mM EDTA, 1.0 mM Na_3VO_4, and a protease inhibitor mixture). Cell lysate protein samples were separated on 10% SDS-PAGE, transferred to nitrocellulose membranes, and immunoblotted with Abs against phospho-ERK, phospho-p38, and phosphor-I$\kappa\beta\alpha$ (Cell Signaling Technology). The blots were also reprobed with Abs against ERK, p38, and I$\kappa\beta\alpha$ proteins (Santa Cruz Biotechnologies) as loading controls.

Monocyte differentiation

PBMC were purified by Ficoll-Hypaque gradient centrifugation (Ficoll-Paque; Pharmacia Biotech AB). Monocytes were adhered to plastic in RPMI 1640 supplemented with glutamine

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

(2 mM), penicillin (100 U/ml), streptomycin (100 μg/ml), and 1% FBS (Omega). After 2 h, nonadherent PBMC were washed off and adherent monocytes were stimulated with 0.1 micrograms/ml anti-LILRA2 or isotype control (mIgG2b, BD Biosciences) and placed on ice for 15 min. Recombinant human GM-CSF at 10 U/ml (Immunex) was then added to the culture and cells were incubated for 2 days in RPMI 1640 supplemented with penicillin, streptomycin, and 10% FBS (Omega). Cells were then harvested and analyzed by flow cytometry and also used as APCs. Cell viability was comparable among all treatment groups. Cytokines and chemokines from cell supernatants were harvested at the time points noted and measured using Searchlight Cytokine Arrays (Pierce Biotechnology). A light microscope photograph was taken of GM-CSF treated cells visualized with a Wright-Giemsa stain (Sigma-Aldrich) according to the manufacturer's instructions.

T cell lines and proliferation assays

T cell lines were derived from skin lesions of leprosy patients as previously described (20, 21). In brief, cells were extracted from lesions with a tissue sieve, and lymphocytes were isolated by density gradient centrifugation. T cell lines were initiated in the presence of irradiated autologous PBMCs and IL-2, followed by culture with HLA-DR-matched APCs or irradiated $CD1^+$ APCs. T cell lines were maintained by serial antigenic stimulation in rIL-2 (1 nM; Chiron Diagnostics)-supplemented medium. Heterologous irradiated PBMCs and PHA were used to propagate T cell lines. For measurement of Ag-specific proliferation, T cells were cultured with irradiated (5000 rad) HLA-DR-matched or heterologous $CD1^+$ APC in culture medium (0.2 ml) in the presence or the absence of bacterial Ags for 3 days in microtiter wells (in triplicate) at 37°C in a 7% CO_2 incubator. Cells were pulsed with [^{3}H]thymidine (1 μCi/well; ICN Biomedicals) and harvested 4–6 h later for liquid scintillation counting. Supernatants were removed from the T cell cultures at 24 h, and IFN-γ was measured by ELISA (IFN-γ; BD Biosciences) according to the manufacturer's instructions. T cells assessed in a secondary stimulation were harvested from primary cultures after 2 days by Ficoll-Hypaque gradient centrifugation and recultured with GM-CSF-stimulated HLA-DR-matched APCs as above for 3 days. Cells were pulsed and harvested for liquid scintillation counting as above.

Results

LILRA2 protein expression in cutaneous leprosy lesions

Immunohistochemistry was performed on biopsy specimens of skin lesions from patients with T-lep and L-lep using mAbs specific for LILRA2. In all samples from L-lep patients, LILRA2 was expressed by 50–80% of the inflammatory cells within granulomas (Fig. 1), while sections labeled with isotype control Ab were negative. In striking contrast, LILRA2-expressing cells in T-lep lesions were rare or absent. The differential frequency of LILRA2 protein expressing cells in leprosy lesions, $LILRA2^+$ cells being more frequent in L-lep vs T-lep lesions, is consistent with the previously described mRNA expression in lesions (10).

Identification of the phenotype of $LILRA2^+$ cells in lepromatous leprosy lesions

To define the phenotype of the $LILRA2^+$ cells observed in L-lep lesions, we labeled the tissues with Abs to the dendritic cell marker CD1b and to the monocyte/macrophage markers CD14 or CD68 (Fig. 2*A*). The expression pattern of LILRA2 in the infiltrate of L-lep skin lesions is similar to that of CD14 and CD68, while very few cells expressed CD1b (<1% positive). Previous studies from our laboratory have shown that L-lep leprosy lesions have significantly fewer $CD1b^+$ cells (22) compared with T-lep lesions. Further studies by double immunofluorescence labeling (Fig. 2*B*) revealed that $LILRA2^+$ cells express the macrophage markers CD14 and CD68, while CD1b does not colocalize with LILRA2. These results identify that LILRA2 is expressed on macrophage-like $CD14^+$, $CD68^+$ cells in L-lep skin lesions.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Effect of anti-LILRA2 Ab on intracellular signaling

LILRs have extracellular regions comprised of two to four C-type Ig domains, while their intracellular domains vary. The proteins are named according to their intracellular domains. Those with long cytoplasmic tails which inhibit cellular activation through recruitment of SHP-1 phosphatase to the ITIM motifs (13,23,24) have a "B" designation and those with short tails have an "A" designation. The "A" members signal via a charged residue in their transmembrane region associated with signaling adaptor molecules containing ITAM motifs, such as FcRIγ (25). The binding of ligands to immunoreceptors triggers the phosphorylation of their ITAM motif via the activation of associated tyrosine kinases. In turn, this allows the recruitment of several intracellular substrates, leading to MAPKs activation.

The capacity of an anti-LILRA2 mouse mAb to mediate cell activation was assessed by testing whether it triggers phosphorylation of ERK and p38 MAPK, mediators of ITAM signaling (26,27). As shown in Fig. 3, p38 and ERK were phosphorylated after 15 min stimulation with anti-LILRA2 Ab but not with the isotype control Ab. I$\kappa\beta\alpha$ phosphorylation was not detected, even after 8 h of stimulation (data not shown). These results indicate that the anti-LILRA2 mouse mAb stimulates intracellular signaling in human peripheral blood monocytes.

Effect of LILRA2 activation on DC differentiation

Because LILRA2$^+$ macrophages predominated in skin lesions from lepromatous patients and inversely correlated with the presence of CD1b$^+$ DCs (22), we hypothesized that LILRA2 signaling may play a down-regulatory role in the monocyte – DC differentiation, a necessary event for the innate immune system to activate the adaptive response.

To establish an in vitro model for studying DC differentiation, we chose to examine monocytes treated for 48 h with recombinant GM-CSF. Although GM-CSF was initially thought to cause the differentiation of monocytes into macrophages (28), a subsequent study indicated that GM-CSF-differentiated monocytes had phenotypic markers of iDC including enhanced Ag presenting function vs medium cells (3). In fact, GM-CSF was identified as a growth factor for DC isolated from human peripheral blood (29). Although DC derived by treatment in vitro of monocytes with both GM-CSF and IL-4 are potent DC and useful for immunotherapy, these cells may not reflect an in vivo population. The GM-CSF and IL-4-derived DC coexpress group I CD1 molecules and CD209 (30), a phenotype not observed in human leprosy skin lesions and activated tonsil (30), lymph node (31), and rheumatoid arthritis synovium (32). Given that GM-CSF alone triggers the differentiation of monocytes into iDC with a cell surface phenotype consistent with that found in situ in human leprosy lesions (30), we chose to study the effect of LILRA2 activation on GM-CSF-treated monocytes.

Monocytes cultured for 2 days with GM-CSF show a DC morphology (Fig. 4*A*) and cell surface phenotype (Fig. 4*B*) with up-regulation of Ag presenting molecules, HLA-DR and CD1b, co-stimulatory molecules, CD80 (B7-1), CD86 (B7-2), and CD40 and CD54 (ICAM-1), and diminished expression of the macrophage markers CD14 (TLR4 coreceptor) (33) and CD16 (FcγRIII) (34,35). This cell surface phenotype is consistent with previous studies (3). Furthermore, GM-CSF treated monocytes have enhanced Ag presentation function (Fig. 4*C*) compared with medium treated cells as demonstrated by their ability to present Ag to CD1b-restricted T cells and HLA-DR-restricted T cells. The morphology, cell surface phenotype and Ag presenting capability of the GM-CSF treated monocytes are consistent with an immature DC phenotype.

To test the hypothesis that LILRA2 activation impairs iDC differentiation, peripheral blood monocytes were treated with GM-CSF and the mouse mAb capable of activating LILRA2. The specificity of LILRA2-activation was demonstrated by use of an isotype control Ab. In the

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

presence of LILRA2-activation, GM-CSF was unable to differentiate monocytes into $CD1b^+$ DC (Fig. 5).

Further phenotypic analysis of iDC markers of GM-CSF-treated monocytes in the presence and absence of LILRA2 activation was performed. GM-CSF induced higher expression of CD1b and HLA-DR (Ag presentation molecule), CD206 (mannose receptor), CD86 (B7.2, costimulatory molecule), and CD40 (T cell costimulation) consistent with an immature DC phenotype. LILRA2 activation prevented the GM-CSF induced up-regulation of these iDC markers as well as the induction of CD1a (Ag presentation molecule) by GM-CSF (data not shown). Instead, LILRA2 activation in the presence of GM-CSF gave rise to cells with a monocyte/macrophage-like phenotype, expressing higher levels of CD16 (FcγRIII), CD32 (FcγRII), and CD64 (FcγRI), as well as CD14 (TLR4 coreceptor) and CD163 (hemoglobin receptor, tissue macrophage marker). To ascertain whether the effects of the anti-LILRA2 Ab could be mediated by its Fc domain via FcRs, stimulation of the monocytes by plate-bound human IgG resulted in a distinctly different phenotype (data not shown). Although LILRA2 and GM-CSF-activated cells expressed cell surface markers characteristic of monocytes and macrophages, their phenotype was unique as these cells did not express significant levels of CD209 (DC-SIGN, macrophage marker), CD23 (FcεRII), or CD36 (scavenger/oxidized LDL receptor), nor did they down-regulate co-stimulatory molecules CD80 (costimulatory molecule, B7-1), CD54 (ICAM-1), and CD58 (LFA-3). This unique phenotype is distinct from IL-4-induced, alternatively activated macrophages that are known to express CD23 (36). These data indicate that LILRA2 activation inhibits the ability of GM-CSF to induce immature DC differentiation, and instead alters differentiation to a monocyte/macrophage-like phenotype.

Effect of LILRA2 activation on Ag presentation

Because LILRA2 activation prevented the differentiation of iDC, we hypothesized that the expression of LILRA2 has a functional significance in host defense against mycobacteria. This hypothesis was tested by measuring the Ag presenting function of LILRA2-activated, GM-CSF-treated monocytes to both CD1b- and MHC class II-restricted T cell lines derived from leprosy lesions. Peripheral blood monocytes were stimulated with GM-CSF as in the previously described experiments, with and without anti-LILRA2 activating Abs or isotype control Abs. Cells were then harvested after 2 days to test their ability to stimulate a proliferative and cytokine response to mycobacteria-reactive T cell lines.

In contrast to the GM-CSF treated monocytes, LILRA2-activated, GM-CSF-treated monocytes were unable to present Ag to a CD1b restricted T cell line CD4.CD1b-LAM3, specific for the glycolipid lipoarabinomannan from *M. leprae* and *M. tuberculosis*. CD1b-restricted lipoarabinomannan-specific T cells were unable to proliferate (Fig. 6*A*) nor produce cytokines (Fig. 6*B*) when stimulated with LILRA2-activated, GM-CSF-treated APCs, while cells receiving an isotype control Ab or GM-CSF alone had robust responses. Because LILRA2 activation prevents GM-CSF induction of CD1b expression (Fig. 5), this result demonstrates the functional relevance of low CD1b expression in terms of Ag presentation to T cells.

To test whether LILRA2 activation can affect MHC class II Ag presentation, a T cell line isolated from a T-lep patient, CD4.DR-GroES2, which is HLA-DR B5– 0101-restricted and specific for a peptide from Gro-ES, a protein Ag from *M. leprae* was studied (Fig. 7). GM-CSF-treated monocytes stimulated these T cell lines to proliferate (Fig. 7, *A* and *B*) as well as produce IFN-γ (Fig. 7, *C* and *D*) in an Ag-specific manner. Adding LILRA2 activation to GM-CSF-treated monocytes markedly diminished their ability to present Ag, while adding an isotype control Ab stimulated robust proliferative and cytokine T cell responses. Both protein (Fig. 7, *A* and *C*) and peptide (Fig. 7, *B* and *D*) Ag presentation was impaired in GM-CSF-treated, LILRA2-activated monocytes, suggesting that the effect of LILRA2 activation was not likely due to altered Ag processing. Phenotypic analysis of the T cells after coculturing

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

with the various APCs showed that they retained their $CD4^+CD25^-FOXP3^-$ phenotype (data not shown) suggesting that the lack of proliferative and IFN-γ response observed was not due to the induction of a T regulatory cell. The unresponsiveness of the T cell lines cocultured with LILRA2-activated, GM-CSF stimulated monocytes (Fig. 8*A*) was transient, as they still proliferated to a subsequent stimulation with GroES peptide-pulsed GM-CSF-treated monocytes (Fig. 8*B*). This suggests LILRA2 activation may promote T cell unresponsiveness without inducing anergy. In summary, LILRA2 activation impedes the ability of the innate immune system to activate the adaptive T cell response, inhibiting both CD1b- and MHC class II-restricted Ag presentation.

Cytokine secretion profile of LILRA2-activated, GM-CSF-stimulated monocytes

Because LILRA2 activation altered the cell surface phenotype of GM-CSF-stimulated monocytes and had such a profound effect on Ag presentation, we hypothesized that these cells may have an altered cytokine secretion profile. Adherent peripheral blood monocytes were stimulated with GM-CSF in the presence or absence of anti-LILRA2-activating Abs or isotype control Abs. Supernatants were then collected after 3, 24, 48, and 72 h and tested for IL-6, IL-8, TNF-α, TGFβ, IL-10, and IL-12 by ELISA. Comparison of the cytokine secretion profiles shows that LILRA2 activation results in increased IL-10, IL-12 p70, TNF-α, IL-6, and IL-8, but has little effect on TGF-β (Fig. 9). These data provide further evidence that LILRA2 activation of GM-CSF-stimulated monocytes results in a cell with altered function.

Discussion

The innate immune system has three distinct roles: a direct antimicrobial response, a proinflammatory (cytokine (or secretory)) response, and an instructive role for an adaptive T cell response. Ag-specific T cells are required for effective host defense against intracellular pathogens including mycobacteria (4,5,37–39). Using leprosy as a model, we demonstrate that LILRA2 activation impairs iDC differentiation and subsequent Ag presentation to both CD1- and MHC class II-restricted T cells. The expression of LILRA2 in leprosy lesions correlated clinically with the progressive form of the disease, characterized by ineffective T cell responses to the pathogen. By down-regulating DC differentiation and function, LILRA2 activation can control the ability of the innate immune system to induce the adaptive T cell response.

The ability of the innate immune response to instruct the adaptive immune response is largely initiated and modulated by DC (2). Although the combination of GM-CSF and IL-4 result in the generation of potent DC that are useful for immunotherapy, we chose to establish a model of iDC by culture with GM-CSF alone. The morphology, cell surface phenotype, and enhanced Ag presenting function of the GM-CSF-treated monocytes show that these cells are consistent with iDC as previously demonstrated (3,30) and correlate with the cell surface phenotype observed in vivo in human leprosy (30). In this study, we identify a regulatory mechanism that alters and prevents iDC differentiation: LILRA2 activation of GM-CSF treated monocytes prevented the up-regulation of CD1b, HLA-DR, CD40, CD86, and CD206, molecules characteristic of iDC and required for efficient Ag presentation to T cells. Consistent with this phenotype, LILRA2 activation of differentiating DC blocked Ag presentation to both CD1b- and MHC class II-restricted T cells. By blocking DC differentiation, LILRA2 activation alters the monocyte's surface phenotype and cytokine secretion profile, and prevents the ability of the innate immune system to stimulate adaptive T cell responses. Differentiation of DCs from peripheral blood monocytes can be influenced by a variety of factors (40) (41,42), for example, "danger signals" (43) activate DCs to differentiate and mature. The regulation of DC differentiation provides a mechanism for regulating Ag presentation capacity to avoid activating T cells in the absence of a threat to the host. Microbial pathogens represent one such threat, and are recognized by the innate immune system as a danger signal via pattern

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

recognition receptors including the TLR family. Mycobacterial lipopeptides activate TLR2/1, to stimulate the differentiation of monocytes to DC by the autocrine induction of GM-CSF (30). Given that TLR activation induces DC differentiation via the release of GM-CSF, LILRA2 activation would likely also inhibit this TLR-induced innate response.

LILRA2 activation induced p38 and ERK phosphorylation, consistent with previous studies that intracellular signaling of ITAMs involves MAPK activation in addition to calcium, PLCγ, and PI-3K (25,44–48). In comparison, ITAMs may counter ITIM signals, as mice lacking an ITIM-containing LILR ortholog have impaired DC differentiation and maturation (49). Clearly, the ability of ITAMs to activate intracellular signals can lead to activation and inhibition of myeloid function (50,50–52). Our data suggest a regulatory role and potential mechanism of ITAM signaling on myeloid function providing a new impetus to further dissect the intracellular crosstalk among signals that activate as well as alter myeloid differentiation and function.

The analysis of LILRA2 expression and activation in leprosy imparts biological relevance to our understanding of human immune responses against microbial infection. In leprosy lesions, LILRA2 was expressed on $CD14^+CD68^+$ macrophage-like cells, more frequent in the progressive lepromatous form of leprosy as compared with the self-limited tuberculoid form of the disease. The local expression of LILRA2 may be regulated by TLR activation, given that TLR2/1 expression is greater in the tuberculoid form (30) and down-regulates LILRA2 expression on monocytes (10). In contrast to the level of LILRA2 expression, the frequency of $CD1^+$ DC in lesions is the direct opposite, lower in the lepromatous form of the disease (22,30).

Although the relatively large numbers of $LILRA2^+$ macrophages and small numbers of $CD1b^+$ DCs in lepromatous lesions (vs tuberculoid) could be explained by comparatively less TLR2/1 expression (53), it is tempting to speculate that LILRA2 activation on monocytes/macrophages additionally blocks iDC differentiation at the site of disease in L-lep and accounts for the low frequency of *M. leprae*-reactive CD1- and MHC class II restricted T cells compared with tuberculoid patients (5,21,54). Consistent with this hypothesis, LILRA2-activated, GM-CSF-treated monocytes were unable to present mycobacterial Ags to CD1- and MHC class II-restricted T cells. Therefore, LILRA2 activation may regulate the ability of the innate immune system to stimulate the effector T cell response, either by inhibiting DC differentiation or by stimulating an alternative differentiation pathway, resulting in a macrophage phenotype with less Ag presenting capacity. The T cell unresponsiveness was not due to the markedly increased IL-10 secretion of LILRA2-activated, GM-CSF-stimulated monocytes, as anti-IL-10 blocking Abs did not recover the Ag presenting capabilities of LILRA2-activated monocytes (data not shown). In this study, we looked at Ag presentation in the context of a memory T cell response, such that further studies are required to examine the activation of naive T cell responses. It would also be important to further characterize the range of functional studies of these LILRA2-activated monocytes.

Although LILRA2 activation altered the ability of GM-CSF-stimulated monocytes to present Ag, it also altered their cytokine secretion profile. In this study, LILRA2-activation of GM-CSF-stimulated monocytes using a mouse mAb stimulated the production of TNF-α, IL-6, IL-8, IL-12p70, and IL-10. Although LILRA2 activation of TLR-stimulated monocytes was shown to decrease IL-12p40 (10) and LILRA2 activation of GM-CSF-stimulated monocytes was shown to increase IL-12p70, LILRA2 activation in both studies caused an increased the IL-10:IL-12 ratio. In addition to showing selective T cell unresponsiveness to mycobacteria, lepromatous leprosy patients also display inflammatory symptoms, termed reactional episodes. The cytokines produced by LILRA2 activation and the potential role of the presence of LILRA2

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

in lepromatous leprosy skin lesions in the pathogenesis of the reactional states of leprosy warrant further study.

The LILRA2$^+$ cells identified in L-lep lesions coexpressed CD14 and CD68, suggesting that they belong to a monocyte/macrophage lineage. However, in vitro data (data not shown) indicated that LILRA2-activated, GM-CSF-treated monocytes have higher levels of CD14 and CD40 as compared with normal peripheral blood monocytes. These data raise the possibility that LILRA2 activation may stimulate an alternative differentiation pathway, with monocytes differentiating into a tissue macrophage phenotype. Because the ligand for LILRA2 has not been identified, the mechanism of activation in leprosy lesions remains uncertain.

A great deal remains to be learned about the LILR family, including identification of all ligands (8,17,24,55–59), immunoregulatory functions, and mechanisms of action. Nevertheless, our data provide insight into a potential regulatory mechanism by which LILRA2 may control the ability of the innate immune system to activate the adaptive T cell response. Although LILRA2 regulation of iDC differentiation may help prevent inappropriate adaptive T cell responses that lead to autoimmunity, the study of human leprosy provides evidence that LILRA2 expression correlates and may contribute to an unfavorable clinical outcome in response to an intracellular pathogen. Genetic profiling of lepromatous and tuberculoid leprosy skin lesions revealed the LILR family members, LILRA2, LILRA3, LILRB2, and LILRB5, were comparatively increased in lesions of lepromatous patients suffering from progressive infection (10). Further studies of the LILR family may define other pathways to regulate innate and adaptive immune responses, providing strategies for intervention in infectious and autoimmune disease.

Acknowledgements

We thank Ryan J. Carbone for his excellent technical assistance, Dr. Philip Liu for many helpful discussions, Dr. Matthew Schibler and the Carol Moss Spivak Cell Imaging Facility in the University of California-Los Angeles Brain Research Institute for the use of the confocal laser microscope, Pierce Biotechnology for measurement of chemokines and cytokines using Searchlight technology, and the University of California-Los Angeles Flow Cytometry Core Laboratory for the use of their facilities.

References

1. Martin-Fontecha A, Sebastiani S, Hopken UE, Uguccioni M, Lipp M, Lanzavecchia A, Sallusto F. Regulation of dendritic cell migration to the draining lymph node: impact on T lymphocyte traffic and priming. J Exp Med 2003;198:615–621. [PubMed: 12925677]
2. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998;392:245–252. [PubMed: 9521319]
3. Sallusto F, Lanzavecchia A. 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 α. J Exp Med 1994;179:1109–1118. [PubMed: 8145033]
4. Ochoa MT, Stenger S, Sieling PA, Thoma-Uszynski S, Sabet S, Cho S, Krensky AM, Rollinghoff M, Nunes SE, Burdick AE, et al. T-cell release of granulysin contributes to host defense in leprosy. Nat Med 2001;7:174–179. [PubMed: 11175847]
5. Sieling PA, Torrelles JB, Stenger S, Chung W, Burdick AE, Rea TH, Brennan PJ, Belisle JT, Porcelli SA, Modlin RL. The human CD1-restricted T cell repertoire is limited to cross-reactive antigens: implications for host responses against immunologically related pathogens. J Immunol 2005;174:2637–2644. [PubMed: 15728470]
6. Ridley DS, Jopling WH. Classification of leprosy according to immunity: a five-group system. Int J Lepr Other Mycobact Dis 1966;34:255–273. [PubMed: 5950347]
7. Samaridis J, Colonna M. Cloning of novel immunoglobulin superfamily receptors expressed on human myeloid and lymphoid cells: structural evidence for new stimulatory and inhibitory pathways. Eur J Immunol 1997;27:660–665. [PubMed: 9079806]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

8. Borges L, Hsu ML, Fanger N, Kubin M, Cosman D. A family of human lymphoid and myeloid Ig-like receptors, some of which bind to MHC class I molecules. J Immunol 1997;159:5192–5196. [PubMed: 9548455]

9. Wagtmann N, Rojo S, Eichler E, Mohrenweiser H, Long EO. A new human gene complex encoding the killer cell inhibitory receptors and related monocyte/macrophage receptors. Curr Biol 1997;7:615–618. [PubMed: 9259559]

10. Bleharski JR, Li H, Meinken C, Graeber TG, Ochoa MT, Yamamura M, Burdick A, Sarno EN, Wagner M, Rollinghoff M, et al. Use of genetic profiling in leprosy to discriminate clinical forms of the disease. Science 2003;301:1527–1530. [PubMed: 12970564]

11. Borges L, Cosman D. LIRs/ILTs/MIRs, inhibitory and stimulatory Ig-superfamily receptors expressed in myeloid and lymphoid cells. Cytokine Growth Factor Rev 2000;11:209–217. [PubMed: 10817964]

12. Arm JP, Nwankwo C, Austen KF. Molecular identification of a novel family of human Ig superfamily members that possess immunoreceptor tyrosine-based inhibition motifs and homology to the mouse gp49B1 inhibitory receptor. J Immunol 1997;159:2342–2349. [PubMed: 9278324]

13. Cella M, Jarrossay D, Facchetti F, Alebardi O, Nakajima H, Lanzavecchia A, Colonna M. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. Nat Med 1999;5:919–923. [PubMed: 10426316]

14. Brown D, Trowsdale J, Allen R. The LILR family: modulators of innate and adaptive immune pathways in health and disease. Tissue Antigens 2004;64:215–225. [PubMed: 15304001]

15. Beckman EM, Melian A, Behar SM, Sieling PA, Chatterjee D, Furlong ST, Matsumoto R, Rosat JP, Modlin RL, Porcelli SA. CD1c restricts responses of mycobacteria-specific T cells: evidence for antigen presentation by a second member of the human CD1 family. J Immunol 1996;157:2795–2803. [PubMed: 8816382]

16. Kim J, Sette A, Rodda S, Southwood S, Sieling PA, Mehra V, Ohmen JD, Oliveros J, Appella E, Higashimoto Y, et al. Determinants of T cell reactivity to the *Mycobacterium leprae* GroES homologue. J Immunol 1997;159:335–343. [PubMed: 9200471]

17. Colonna M, Navarro F, Bellon T, Llano M, Garcia P, Samaridis J, Angman L, Cella M, Lopez-Botet M. A common inhibitory receptor for major histocompatibility complex class I molecules on human lymphoid and myelomonocytic cells. J Exp Med 1997;186:1809–1818. [PubMed: 9382880]

18. Behar SM, Porcelli SA, Beckman EM, Brenner MB. A pathway of costimulation that prevents anergy in CD28-T cells: B7-independent co-stimulation of CD1-restricted T cells. J Exp Med 1995;182:2007–2018. [PubMed: 7500046]

19. Modlin RL, Hofman FM, Horwitz DA, Husmann LA, Gillis S, Taylor CR, Rea TH. In situ identification of cells in human leprosy granulomas with monoclonal antibodies to interleukin 2 and its receptor. J Immunol 1984;132:3085–3090. [PubMed: 6327818]

20. Sieling PA, Chatterjee D, Porcelli SA, Prigozy TI, Mazzaccaro RJ, Soriano T, Bloom BR, Brenner MB, Kronenberg M, Brennan PJ. CD1-restricted T cell recognition of microbial lipoglycan antigens. Science 1995;269:227–230. [PubMed: 7542404]

21. Mehra V, Bloom BR, Bajardi AC, Grisso CL, Sieling PA, Alland D, Convit J, Fan XD, Hunter SW, Brennan PJ. A major T cell antigen of *Mycobacterium leprae* is a 10-kD heat-shock cognate protein. J Exp Med 1992;175:275–284. [PubMed: 1730920]

22. Sieling PA, Jullien D, Dahlem M, Tedder TF, Rea TH, Modlin RL, Porcelli SA. CD1 expression by dendritic cells in human leprosy lesions: correlation with effective host immunity. J Immunol 1999;162:1851–1858. [PubMed: 9973451]

23. Burshtyn DN, Scharenberg AM, Wagtmann N, Rajagopalan S, Berrada K, Yi T, Kinet JP, Long EO. Recruitment of tyrosine phosphatase HCP by the killer cell inhibitor receptor. Immunity 1996;4:77–85. [PubMed: 8574854]

24. Fanger NA, Cosman D, Peterson L, Braddy SC, Maliszewski CR, Borges L. The MHC class I binding proteins LIR-1 and LIR-2 inhibit Fc receptor-mediated signaling in monocytes. Eur J Immunol 1998;28:3423–3434. [PubMed: 9842885]

25. Nakajima H, Samaridis J, Angman L, Colonna M. Human myeloid cells express an activating ILT receptor (ILT1) that associates with Fc receptor γ-chain. J Immunol 1999;162:5–8. [PubMed: 9886363]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

26. Werlen G, Hausmann B, Palmer E. A motif in the $\alpha\gamma$ T-cell receptor controls positive selection by modulating ERK activity. Nature 2000;406:422–426. [PubMed: 10935640]
27. Hesslein DG, Takaki R, Hermiston ML, Weiss A, Lanier LL. Dysregulation of signaling pathways in CD45-deficient NK cells leads to differentially regulated cytotoxicity and cytokine production. Proc Natl Acad Sci USA 2006;103:7012–7017. [PubMed: 16627620]
28. Young DA, Lowe LD, Clark SC. Comparison of the effects of IL-3, granulocyte-macrophage colony-stimulating factor, and macrophage colony-stimulating factor in supporting monocyte differentiation in culture: analysis of macrophage antibody-dependent cellular cytotoxicity. J Immunol 1990;145:607–615. [PubMed: 2142182]
29. Markowicz S, Engleman EG. Granulocyte-macrophage colony-stimulating factor promotes differentiation and survival of human peripheral blood dendritic cells in vitro. J Clin Invest 1990;85:955–961. [PubMed: 2179270]
30. Krutzik SR, Tan B, Li H, Ochoa MT, Liu PT, Sharfstein SE, Graeber TG, Sieling PA, Liu YJ, Rea TH, et al. TLR activation triggers the rapid differentiation of monocytes into macrophages and dendritic cells. Nat Med 2005;11:653–660. [PubMed: 15880118]
31. Granelli-Piperno A, Pritsker A, Pack M, Shimeliovich I, Arrighi JF, Park CG, Trumpfheller C, Piguet V, Moran TM, Steinman RM. Dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin/CD209 is abundant on macrophages in the normal human lymph node and is not required for dendritic cell stimulation of the mixed leukocyte reaction. J Immunol 2005;175:4265–4273. [PubMed: 16177066]
32. van Lent PL, Figdor CG, Barrera P, van Ginkel K, Sloetjes A, van den Berg WB, Torensma R. Expression of the dendritic cell-associated C-type lectin DC-SIGN by inflammatory matrix metalloproteinase-producing macrophages in rheumatoid arthritis synovium and interaction with intercellular adhesion molecule 3-positive T cells. Arthritis Rheum 2003;48:360–369. [PubMed: 12571844]
33. Goyert SM, Ferrero E, Rettig WJ, Yenamandra AK, Obata F, Le Beau MM. The CD14 monocyte differentiation antigen maps to a region encoding growth factors and receptors. Science 1988;239:497–500. [PubMed: 2448876]
34. Lee SH, Crocker PR, Westaby S, Key N, Mason DY, Gordon S, Weatherall DJ. Isolation and immunocytochemical characterization of human bone marrow stromal macrophages in hemopoietic clusters. J Exp Med 1988;168:1193–1198. [PubMed: 3049905]
35. Baumgartner I, Scheiner O, Holzinger C, Boltz-Nitulescu G, Klech H, Lassmann H, Rumpold H, Forster O, Kraft D. Expression of the VEP13 antigen (CD16) on native human alveolar macrophages and cultured blood monocytes. Immunobiology 1988;177:317–326. [PubMed: 2971605]
36. Becker S, Daniel EG. Antagonistic and additive effects of IL-4 and interferon-γ on human monocytes and macrophages: effects on Fc receptors, HLA-D antigens, and superoxide production. Cell Immunol 1990;129:351–362. [PubMed: 2166625]
37. Serbina NV, Lazarevic V, Flynn JL. $CD4^+$ T cells are required for the development of cytotoxic $CD8^+$ T cells during *Mycobacterium tuberculosis* infection. J Immunol 2001;167:6991–7000. [PubMed: 11739519]
38. Caruso AM, Serbina N, Klein E, Triebold K, Bloom BR, Flynn JL. Mice deficient in CD4 T cells have only transiently diminished levels of IFN-γ, yet succumb to tuberculosis. J Immunol 1999;162:5407–5416. [PubMed: 10228018]
39. Salgame P. Host innate and Th1 responses and the bacterial factors that control *Mycobacterium tuberculosis* infection. Curr Opin Immunol 2005;17:374–380. [PubMed: 15963709]
40. Sallusto F, Cella M, Danieli C, Lanzavecchia A. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. J Exp Med 1995;182:389–400. [PubMed: 7629501]
41. Reddy A, Sapp M, Feldman M, Subklewe M, Bhardwaj N. A monocyte conditioned medium is more effective than defined cytokines in mediating the terminal maturation of human dendritic cells. Blood 1997;90:3640–3646. [PubMed: 9345048]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

42. Romani N, Reider D, Heuer M, Ebner S, Kampgen E, Eibl B, Niederwieser D, Schuler G. Generation of mature dendritic cells from human blood: an improved method with special regard to clinical applicability. J Immunol Methods 1996;196:137–151. [PubMed: 8841452]
43. Matzinger P. The danger model: a renewed sense of self. Science 2002;296:301–305. [PubMed: 11951032]
44. Samelson LE. Signal transduction mediated by the T cell antigen receptor: the role of adapter proteins. Annu Rev Immunol 2002;20:371–394. [PubMed: 11861607]
45. McVicar DW, Burshtyn DN. Intracellular signaling by the killer immunoglobulin-like receptors and Ly49. Sci STKE 2001;2001:RE1. [PubMed: 11752646]
46. Isakov N. ITAMs: immunoregulatory scaffolds that link immunoreceptors to their intracellular signaling pathways. Recept Channels 1998;5:243–253. [PubMed: 9666518]
47. Humphrey MB, Lanier LL, Nakamura MC. Role of ITAM-containing adapter proteins and their receptors in the immune system and bone. Immunol Rev 2005;208:50–65. [PubMed: 16313340]
48. Hamerman JA, Lanier LL. Inhibition of immune responses by ITAM-bearing receptors. Sci STKE 2006;2006:RE1. [PubMed: 16449667]
49. Ujike A, Takeda K, Nakamura A, Ebihara S, Akiyama K, Takai T. Impaired dendritic cell maturation and increased TH2 responses in PIR-B$^{-/-}$ mice. Nat Immunol 2002;3:542–548. [PubMed: 12021780]
50. Hamerman JA, Jarjoura JR, Humphrey MB, Nakamura MC, Seaman WE, Lanier LL. Cutting edge: inhibition of TLR and FcR responses in macrophages by triggering receptor expressed on myeloid cells TREM-2 and DAP12. J Immunol 2006;177:2051–2055. [PubMed: 16887962]
51. Bakker AB, Baker E, Sutherland GR, Phillips JH, Lanier LL. Myeloid DAP12-associating lectin (MDL)-1 is a cell surface receptor involved in the activation of myeloid cells. Proc Natl Acad Sci USA 1999;96:9792–9796. [PubMed: 10449773]
52. Bakker AB, Hoek RM, Cerwenka A, Blom B, Lucian L, McNeil T, Murray R, Phillips LH, Sedgwick JD, Lanier LL. DAP12-deficient mice fail to develop autoimmunity due to impaired antigen priming. Immunity 2000;13:345–353. [PubMed: 11021532]
53. Krutzik SR, Ochoa MT, Sieling PA, Uematsu S, Ng YW, Legaspi A, Liu PT, Cole ST, Godowski PJ, Maeda Y, et al. Activation and regulation of toll-like receptors 2 and 1 in human leprosy. Nat Med 2003;9:525–532. [PubMed: 12692544]
54. Modlin RL, Melancon-Kaplan J, Young SM, Pirmez C, Kino H, Convit J, Rea TH, Bloom BR. Learning from lesions: patterns of tissue inflammation in leprosy. Proc Natl Acad Sci USA 1988;85:1213–1217. [PubMed: 3257577]
55. Allan DS, Colonna M, Lanier LL, Churakova TD, Abrams JS, Ellis SA, McMichael AJ, Braud VM. Tetrameric complexes of human histocompatibility leukocyte antigen (HLA)-G bind to peripheral blood myelomonocytic cells. J Exp Med 1999;189:1149–1156. [PubMed: 10190906]
56. Chapman TL, Heikeman AP, Bjorkman PJ. The inhibitory receptor LIR-1 uses a common binding interaction to recognize class I MHC molecules and the viral homolog UL18. Immunity 1999;11:603–613. [PubMed: 10591185]
57. Colonna M, Samaridis J, Cella M, Angman L, Allen RL, O'Callaghan CA, Dunbar R, Ogg GS, Cerundolo V, Rolink A. Human myelomonocytic cells express an inhibitory receptor for classical and nonclassical MHC class I molecules. J Immunol 1998;160:3096–3100. [PubMed: 9531263]
58. Cosman D, Fanger N, Borges L, Kubin M, Chin W, Peterson L, Hsu ML. A novel immunoglobulin superfamily receptor for cellular and viral MHC class I molecules. Immunity 1997;7:273–282. [PubMed: 9285411]
59. Allen RL, Raine T, Haude A, Trowsdale J, Wilson MJ. Leukocyte receptor complex-encoded immunomodulatory receptors show differing specificity for alternative HLA-B27 structures. J Immunol 2001;167:5543–5547. [PubMed: 11698424]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

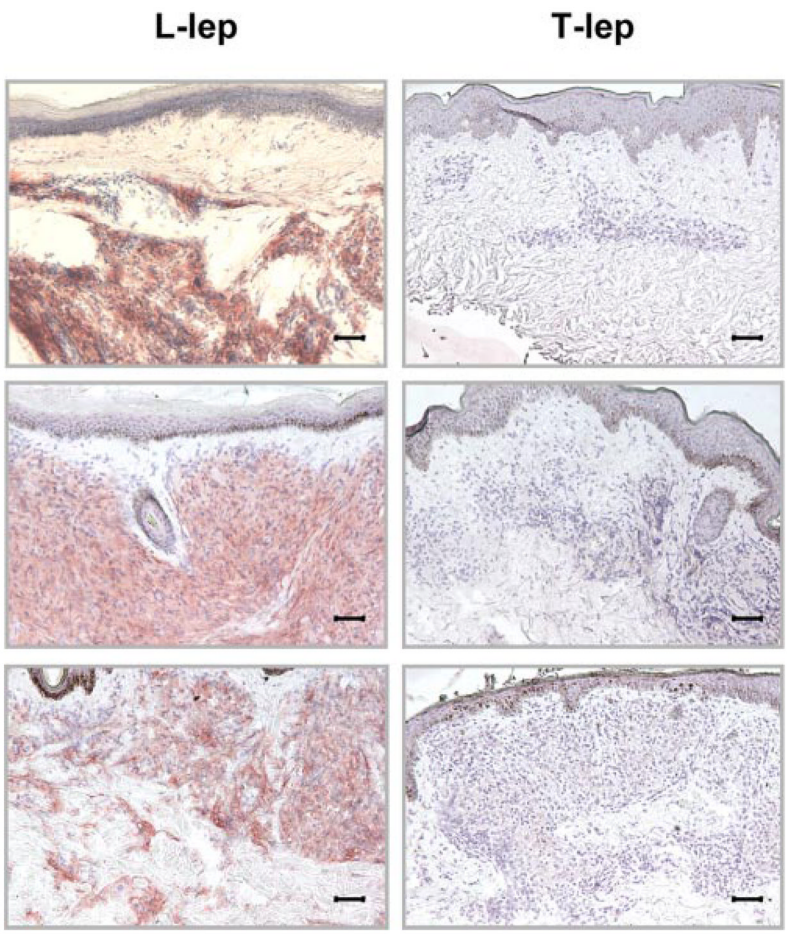

FIGURE 1.

LILRA2 expression in leprosy skin lesions in vivo. LILRA2 expression in skin lesions from three patients with lepromatous and three with tuberculoid leprosy. Thin (4 μM) sections of leprosy biopsy samples were incubated with anti-LILRA2 and stained secondarily with an immunoperoxidase method followed by counterstaining with hematoxylin. The isotype controls were negative. The findings shown are representative of five patients in each group. Photographs were taken using 10× objective lens. Each bar denotes 50 microns.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

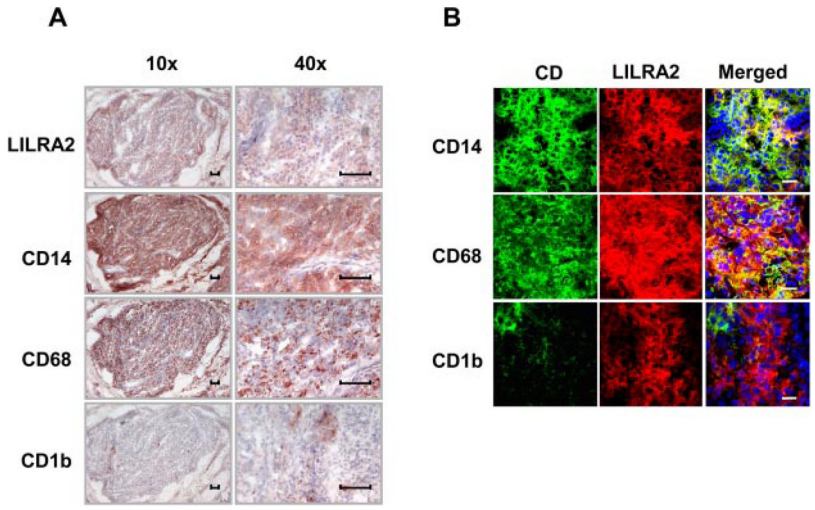

FIGURE 2.

Phenotype of LILRA2$^+$ cells in leprosy skin lesions. *A*, Immunoperoxidase labeling of L-lep skin sections using mAbs specific for LILRA2, CD14, CD68 and CD1b expression. This trend was observed in three of three patients examined. Photographs were taken using the objective lenses noted in the figure. Each bar denotes 50 microns. *B*, Immunofluorescence confocal images from L-lep skin lesions. Skin lesions from L-lep patients were sectioned and labeled with specific Abs and visualized using confocal laser microscopy. Images were photographed using a 63× objective. Each bar denotes 20 microns.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

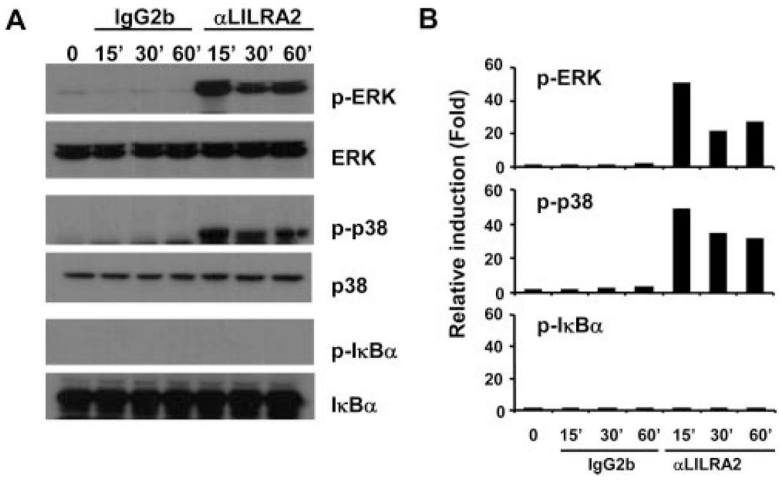

FIGURE 3.

Anti-LILRA2 Ab leads to intracellular signaling in monocytes. PBMC were stimulated with anti-LILRA2 or isotype control Abs. *A*, Western blot analysis of phosphorylated ERK, p38, and I*κβα* were performed at the time points indicated. The blots were also reprobed with Abs against ERK protein, p38 protein and I*κβα* protein, respectively, as loading controls. *B*, Quantification by densitometry of the relative intensities of phosphorylated proteins shown in *A*. Blots of phosphorylated ERK, p38, and I*κβα* were quantitated, and the band intensities were normalized to corresponding total protein level. Data are graphed as fold induction relative to the untreated sample in each experiment. This result is representative of two independent experiments.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

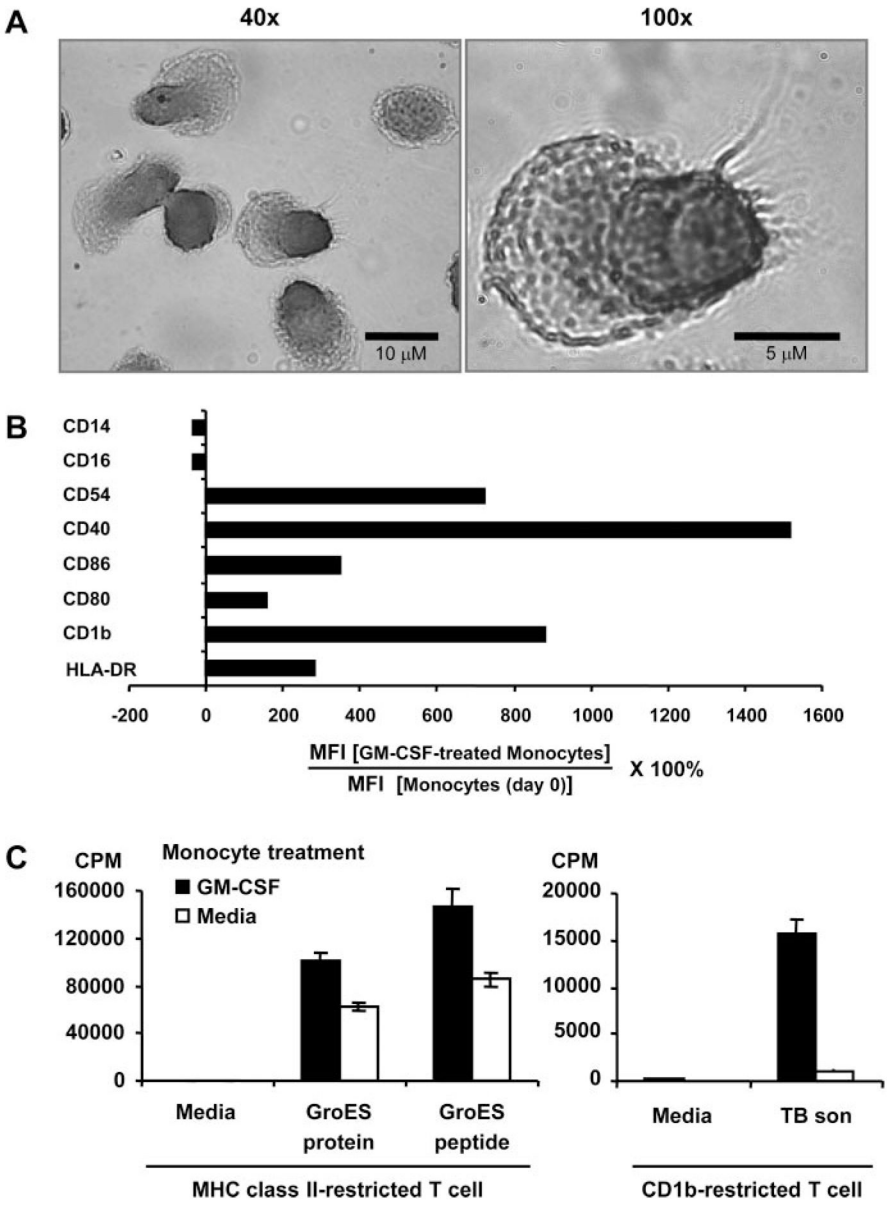

FIGURE 4.

Characterization of GM-CSF-treated monocytes. *A*, Photographs of monocytes stimulated with GM-CSF for 2 days were taken using the objective lenses noted in the figure. *B*, Cell surface phenotype of GM-CSF-treated monocytes after 2 days compared with monocytes at time 0. *C*, Ag presentation function of GM-CSF treated vs untreated monocytes. Proliferative responses of a MHC class II (HLA-DR B5– 0101)-restricted T cell line (CD4.DR-GroES2) to 0.1 μg/ml Gro-ES protein or 0.03 μM Gro-ES peptide and a CD1b-restricted T cell line (CD4.CD1b-LAM3) to 1 μg/ml *M. tuberculosis* sonicate. Error bars represent ± 1 SEM. This result is representative of three independent experiments.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

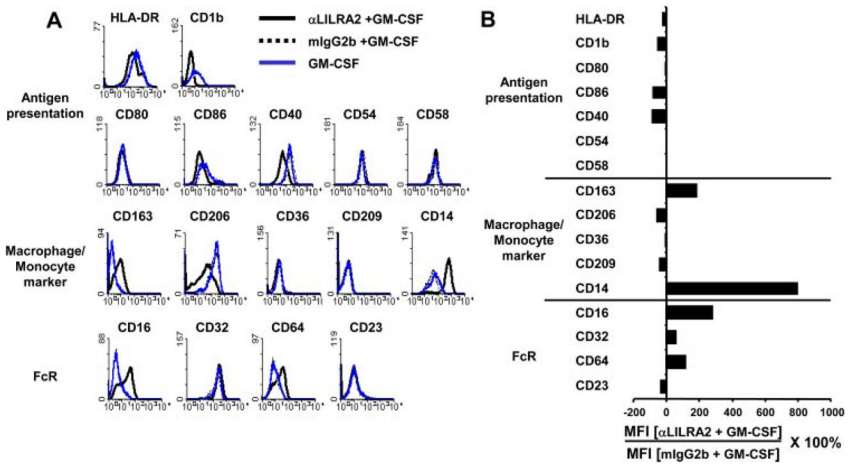

FIGURE 5.

LILRA2 activation alters the cell surface phenotype of GM-CSF-stimulated monocytes. *A*, Monocytes stimulated with GM-CSF (blue lines) were compared with those treated with anti-LILRA2 mAb and GM-CSF (black lines) or isotype control mIgG2b and GM-CSF (dashed lines). For most cell surface markers, the histograms for cells treated with isotype control mIgG2b (dashed lines) followed by GM-CSF overlay the histograms for cells treated with GM-CSF alone (blue lines). Flow cytometric data are shown as histograms of each individual cell surface marker. *B*, Mean fluorescence intensity (MFI) of each histogram in (*A*) for LILRA2-activated, GM-CSF-treated monocytes is shown as a percentage of the MFI for isotype control-treated cells for each cell surface marker. Data shown are representative of three to five experiments.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

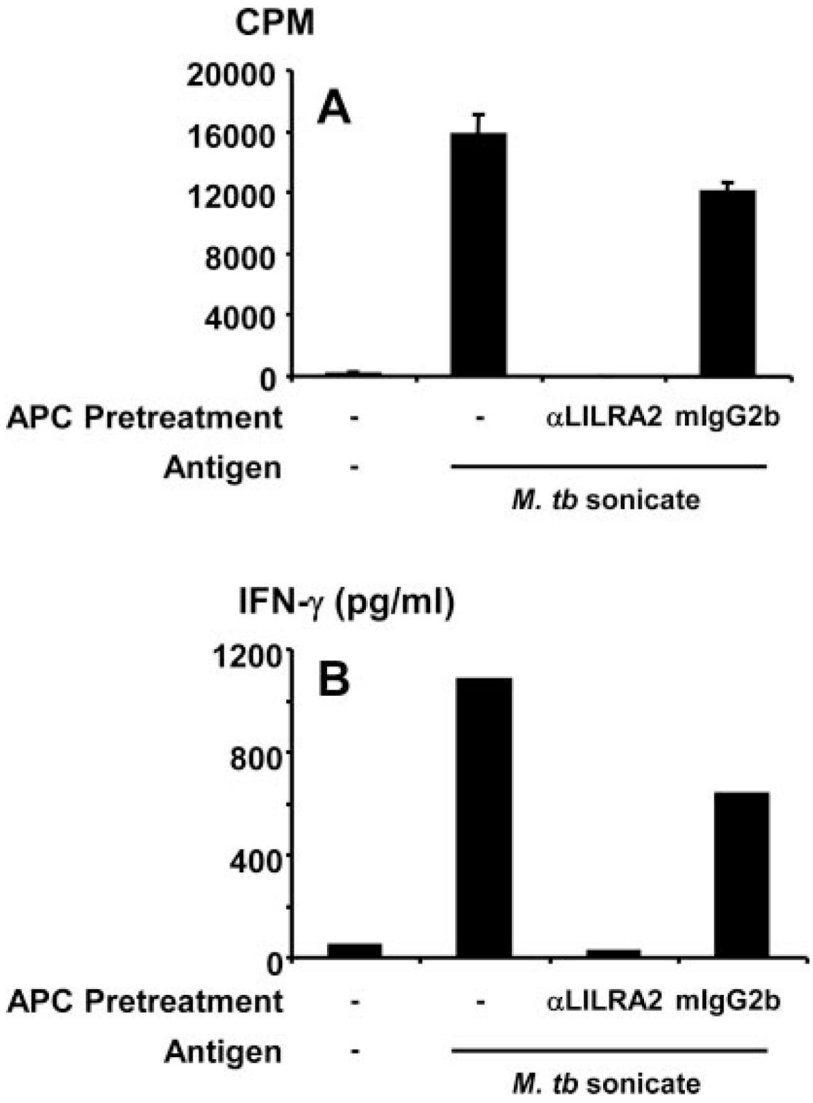

FIGURE 6.

CD1b-Ag presentation by GM-CSF-stimulated monocytes. Proliferative (*A*) and IFN-γ (*B*) responses of a CD1b-restricted T cell line (CD4.CD1b-LAM3) to 1 μg/ml *M. tuberculosis* sonicate presented by monocytes stimulated with GM-CSF ± an anti-LILRA2 or isotype control Ab. Error bars show ± 1 SEM. IFN-γ was measured by ELISA. Data shown are representative of experiments using monocytes from 2 of 2 donors.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

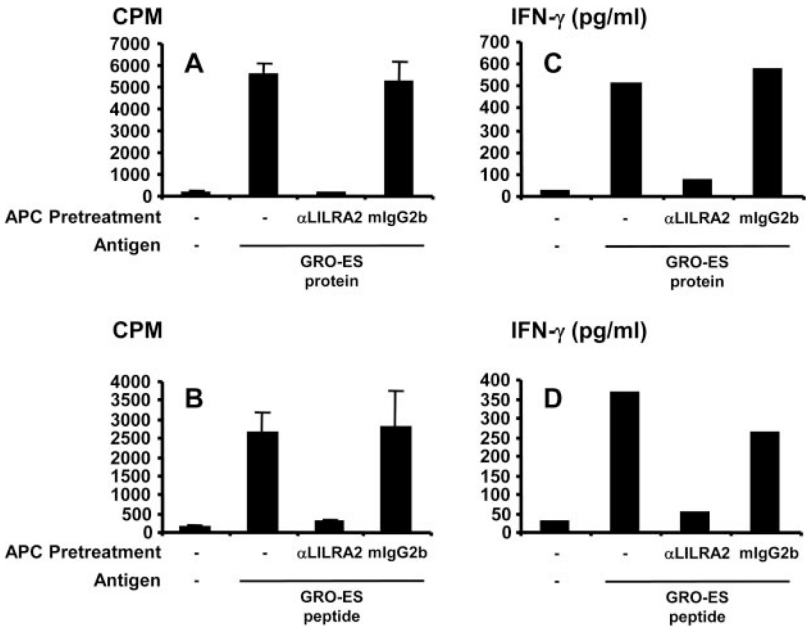

FIGURE 7.

MHC class II-Ag presentation by GM-CSF stimulated monocytes treated as in Fig. 6. Proliferative (*A* and *B*) and IFN-γ (*C* and *D*) responses of a HLA-DR B5– 0101-restricted T cell line (CD4.DR-GroES2) to 0.03 μM of GRO-ES protein (*A* and *C*) or peptide (*B* and *D*). Error bars show ± 1 SEM. IFN-γ was measured by ELISA. Data shown are representative of experiments using monocytes from 3 of 3 donors.

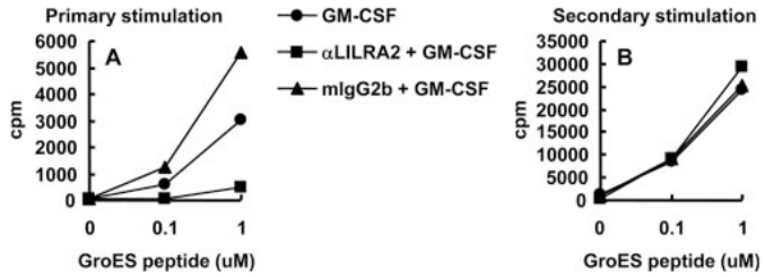

FIGURE 8.

T cell line responsiveness to subsequent MHC class II-Ag presentation by GM-CSF stimulated monocytes after initial presentation assays as in Fig. 7. *A*, Proliferative responses of a HLA-DR B5– 0101-restricted T cell line (CD4.DR-GroES2) to GRO-ES peptide presented by monocytes stimulated with GM-CSF (circles) ± an anti-LILRA2 (squares) or isotype control (triangles) Ab. *B*, Proliferative responses of T cells recovered after 2 days' culture from experiment in (*A*) to GRO-ES peptide presented by monocytes stimulated with GM-CSF.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

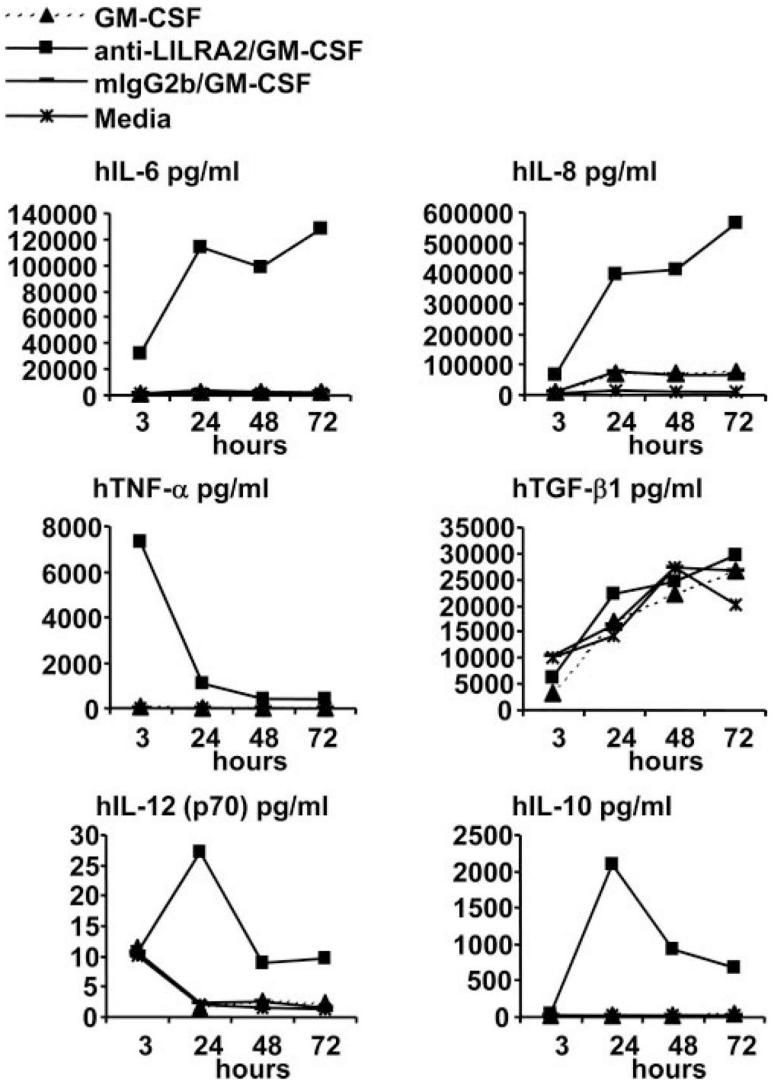

FIGURE 9.

Time course of cytokine secretion patterns of GM-CSF-stimulated monocytes. Cytokines were measured from supernatants harvested at 3, 24, 48, and 72 h from monocytes stimulated with GM-CSF (triangles), anti-LILRA2 mAb and GM-CSF (squares), isotype control mIgG2b and GM-CSF (bold dashed lines), or cultured in medium alone (asterisks). Cytokine and chemokine levels were measured by ELISA (Searchlight Arrays).