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Interleukin-1β triggers the differentiation of macrophages with enhanced capacity to present mycobacterial antigen to T cells

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Summary

The rapid differentiation of monocytes into macrophages (MΦ) and dendritic cells is a pivotal aspect of the innate immune response. Differentiation is triggered following recognition of microbial ligands that activate pattern recognition receptors or directly by pro-inflammatory cytokines. We demonstrate that interleukin-1β (IL-1β) induces the rapid differentiation of monocytes into CD209$^+$ MΦ, similar to activation via Toll-like receptor 2/1, but with distinct phenotypic and functional characteristics. The IL-1β induced MΦ express higher levels of key markers of phagocytosis, including the Fc-receptors CD16 and CD64, as well as CD36, CD163 and CD206. In addition, IL-1β-induced MΦ exert potent phagocytic activity towards inert particles, oxidized low-density lipoprotein and mycobacteria. Furthermore, IL-1β-induced MΦ express higher levels of HLA-DR and effectively present mycobacterial antigens to T cells. Therefore, the ability of IL-1β to induce monocyte differentiation into MΦ with both phagocytosis and antigen-presenting function is a distinct part of the innate immune response in host defence against microbial infection.

Keywords: antigen presentation; infection; innate immunity; macrophages/monocytes; mycobacteria/*Mycobacterium*.

Introduction

At sites of infection, monocytes are recruited from the blood and differentiate *in situ* into diverse macrophage (MΦ) and dendritic cell (DC) populations as part of the local innate immune response. The differentiation of monocytes into MΦ and DC can be triggered by microbial ligands that activate pattern recognition receptors, resulting in the release of cytokines that induce specific functional programmes. For example, Toll-like receptor (TLR) activation of human monocytes induces rapid differentiation into two distinct subsets: MΦ and DC, involving the up-regulation of cytokine and receptor pairs.[1] The TLR induction of interleukin-15 (IL-15) was required and also sufficient for differentiation of monocytes into MΦ, which were CD209$^+$ and co-expressed the MΦ markers, CD14, CD16, CD64 but not the DC marker CD1b. In addition, TLR induction of granulocyte–macrophage colony-stimulating factor (GM-CSF) was required and also sufficient for differentiation of CD1b$^+$ DC; these cells co-expressed high levels of the DC markers CD1b, CD40 and CD86 but not the MΦ markers. Alternatively, NOD2-mediated induction of IL-32 was required and also sufficient to trigger a distinct DC pathway.[2] These pattern recognition receptor- and cytokine-induced MΦ and DC pathways were found to contribute to host responses against microbial infection in humans.

A key cytokine of the innate immune response to microbial infection is Interleukin-1β (IL-1β), a pro-inflammatory cytokine that triggers monocyte activation, inducing cytokine release and differentiation into DC.[3] IL-1β can directly trigger monocyte differentiation into CD1b$^+$ DC[3] and can act with immune complexes to induce differentiation of M2 MΦ.[4] The activation of

monocytes with IL-1β and TLR2/1 ligands (TLR2/1L) is transduced via a common MyD88 signalling pathway.[5] Given that TLR2/1 activation triggers the differentiation of monocytes into MΦ and DC, we hypothesized that IL-1β might also trigger monocyte differentiation into specific cell types with distinct innate immune functions.

Materials and methods

Microbial ligands and cytokines

For activation of monocytes, recombinant human IL-1β (10 ng/ml, R&D Systems, Minneapolis, MN), the TLR2/1L, mycobacterial 19 000 molecular weight lipopeptide (1 μg/ml, EMC Microcollections, Tübingen, Germany),[1] recombinant human GM-CSF (1 U/ml, Genzyme, Cambridge, MA) or IL-15 (200 ng/ml, R&D Systems) was used. These reagents were all tested for endotoxin by *Limulus* amoebocyte lysate assay (Lonza, Basel, Switzerland) to be endotoxin free (detection limit < 0·1 EU/ml).

Monocyte isolation and enrichment

We obtained whole blood from healthy donors (UCLA I.R.B. #92-10-591-31) with informed consent. Peripheral blood mononuclear cells were isolated using Ficoll (GE Healthcare, Little Chalfont, UK) gradient centrifugation and monocytes were further enriched using Percoll density gradient (GE Healthcare) and subsequent adherence in 1% fetal calf serum (FCS) for 2 hr. Monocyte purity was found to be > 80% as measured by CD14 expression. Cells were cultured for 2 or 3 days in RPMI-1640 and 10% FCS (Omega Scientific, Yately, UK). To purify $CD209^+$ MΦ, cells were cultured with IL-1β or TLR2/1L for 2 days in RPMI-1640 with 10% FCS (Omega Scientific) and labelled with a magnetic microbead-coupled CD209 antibody (Miltenyi Biotec, Bergisch Gladbach, Germany). The positive population was collected using magnetic-activated cell sorting according to the manufacturer's recommendations (Miltenyi Biotec). Purity was confirmed to be > 90% by flow cytometry.

Cell surface labelling and FACS

Cell surface expression of antigenic determinants was measured using epitope-specific antibodies and cells were acquired and analysed as described elsewhere.[6] The following fluorochrome-coupled monoclonal antibodies were used: CD23, CD64, CD16, SRAI/II, MARCO, CD36, CD163, CD206, CD86, CD80, CD40, HLA-DR and CD209 (BD Pharmingen, Franklin Lakes, NJ). For detection of CD1b a monoclonal primary antibody (1 μg/ml, Bcd3.1, American Type Culture Collection, Manassass, VA) was used, followed by an IgG1-specific fluorochrome-coupled secondary antibody (Invitrogen, Carlsbad, CA).

Endocytosis and phagocytosis assays

Interleukin-1β- or TLR2/1L-derived MΦ were incubated with the following: Lucifer yellow or DiI(1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate)-labelled $CuSO_4$-oxidized low-density lipoprotein (oxLDL, Intracel RP-175) or live mycobacteria, bacillus Calmette–Guérin-green fluorescent protein (BCG-GFP; gift from Dr Barry Bloom, Harvard, Cambridge, MA) as previously described.[1,7,8] Binding was assayed by incubating macrophages with Lucifer yellow, oxLDL or BCG-GFP at 4°. Cells were then harvested, labelled with CD209, and analysed by flow cytometry. Intracellular uptake was shown as $\Delta MFI = MFI_{uptake} - MFI_{binding}$, where MFI is mean fluorescence intensity. Cells were then harvested, labelled with CD209, and analysed by flow cytometry.

T-cell assays

For investigation of MHC class II restricted antigen presentation to T cells, monocytes from HLA-DR-matched donors were differentiated and subsequently purified using IL-1β, TLR2/1L or GM-CSF (as described above). The $CD209^+$ MΦ and $CD1b^+$ DC were then cultured with the MHC class II-restricted T-cell clone derived from a patient with tuberculoid leprosy (1×10^5, BCD4.9) that recognized the *Mycobaterium leprae* GroES protein and a defined peptide spanning amino acids 28–39 in an HLA-DR15-restricted manner as described previously.[9] Interferon-γ (IFN-γ) was measured by ELISA (BD Pharmingen) and proliferation was measured using $[^3H]$thymidine incorporation as described elsewhere.[10]

Results

IL-1β induces rapid differentiation of monocytes into $CD209^+$ MΦ

To determine whether IL-1β induced monocyte differentiation into MΦ, we first performed a dose–response experiment measuring IL-1β induction of CD209. A dose–response was obtained for the induction of CD209 expression with the maximum response observed at 10 ng/ml IL-1β (see Supplementary material, Fig. S1), the dose selected for further experiments. The optimal concentrations for IL-15-, GM-CSF- and TLR2/1L-induced monocyte differentiation were determined previously in our laboratory.[1] We next compared the effect of IL-1β versus a synthetic TLR2/1L, the 19 000 molecular weight *Mycobacterium tuberculosis*-derived lipopeptide, on monocyte differentiation (Fig. 1a; see Supplementary material, Fig. S2). We found that after 2 days, IL-1β induced CD209-expressing MΦ (37% of myeloid gate) compared with cells cultured in medium alone (3·6% of myeloid gate), but did not induce significant levels of $CD1b^+$ DC

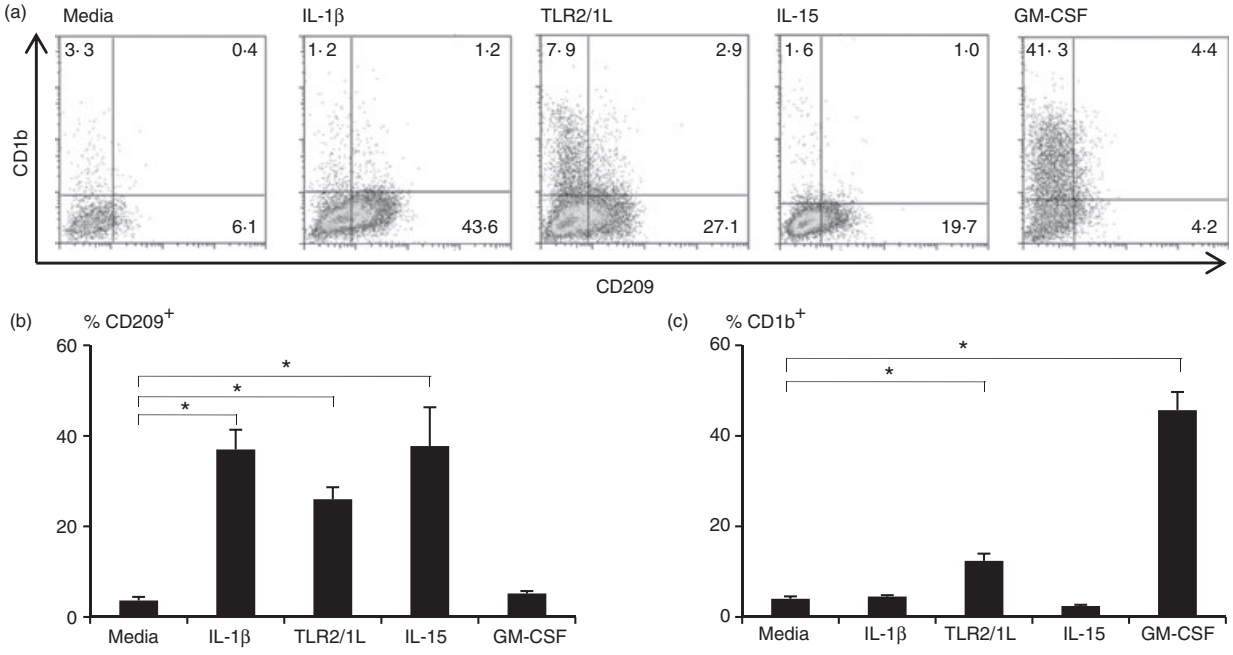

Figure 1. Interleukin-1β (IL-1β) induces monocyte differentiation into CD209$^+$ macrophages (MΦ). Human peripheral blood monocytes were stimulated with either IL-1β, toll-like receptor (TLR) 2/1L, IL-15 or granulocyte–macrophage colony-stimulating factor (GM-CSF) as indicated. After day 2 the cells were analysed by flow cytometry for the expression of CD209 and CD1b. (a) A representative double labelling for CD1b and CD209 is shown for each condition. (b, c) Percentage of CD209$^+$ MΦ and CD1b$^+$ dendritic cells (DC) are indicated as mean $\pm$ SEM; $n = 7$. Statistical significance was calculated by two-tailed Student's *t*-test.

(4·5% versus 4% by media alone) (Fig. 1b,c). The MFI of the CD209$^+$ cells induced by IL-1β and TLR2/1L were not significantly different. After 3 days of culture, IL-1β induced CD1b$^+$ DC (see Supplementary material, Fig. S2) consistent with previous results,[3] whereas the number of CD209$^+$ cells did not significantly change. As a positive control, TLR2/1L induced distinct populations of both CD209$^+$ MΦ (26%) and CD1b$^+$ DC (12·4%) after 2 days of culture (Fig. 1b,c).[1] Given that TLR2/1L induces CD209$^+$ MΦ and CD1b$^+$ DC via IL-15 and GM-CSF, respectively, we compared the effect of these two cytokines on monocyte differentiation. After 2 days of culture, IL-15 induced CD209$^+$ MΦ (37·8%), but not CD1b$^+$ DC (2·4%); whereas, GM-CSF induced CD1b$^+$ DC (45·7%), but not CD209$^+$ MΦ (5·2%). These data indicate that IL-1β is a more potent inducer of monocyte differentiation into CD209$^+$ MΦ than TLR2/1L.

IL-1β-induced CD209$^+$ MΦ differ phenotypically from those induced by TLR2/1 activation

Since CD209$^+$ MΦ are induced by either IL-1β or TLR2/1L, we wanted to compare the phenotype of these MΦ populations according to cell surface determinants. In these experiments, we gated on the CD209$^+$ cells when measuring other cell surface markers. The IL-1β-induced CD209$^+$ MΦ expressed at least 1·6-fold higher percentage positive cells and 2·5-fold or higher MFI levels of CD16 (Fcγ receptor III; FcγRIII) and CD64 (FcγRI) than TLR2/1L-induced MΦ (Fig. 2). Furthermore, IL-1β induced at least a twofold higher percentage of cells expressing key phagocytic markers, such as the SRAI/II (scavenger receptors), MARCO (macrophage receptor with a collagenous structure), CD36 (scavenger receptor for oxLDL), CD163 (haemoglobin/haptoglobin scavenger receptor) and CD206 (MΦ mannose receptor). Furthermore, the expression levels of CD36, CD163 and CD206 were also significantly higher in IL-1β-induced versus TLR2/1L-induced MΦ. Finally, IL-1β induced about fourfold higher expression levels of the antigen presentation molecule HLA-DR compared with TLR2/1L. Therefore, our data indicate that IL-1β-induced MΦ express higher levels of cell surface proteins involved in phagocytosis and antigen presentation than TLR2/1L-induced MΦ.

IL-1β-induced CD209$^+$ MΦ show high phagocytic activity

The high expression of markers for phagocytosis on the IL-1β-induced CD209$^+$ MΦ suggests that these cells have a potential for phagocytic activity. To measure the potential for pinocytosis and phagocytosis, we tested differentiated MΦ for the specific uptake of Lucifer yellow, oxLDL and BCG. We also compared uptake by IL-15-derived MΦ,

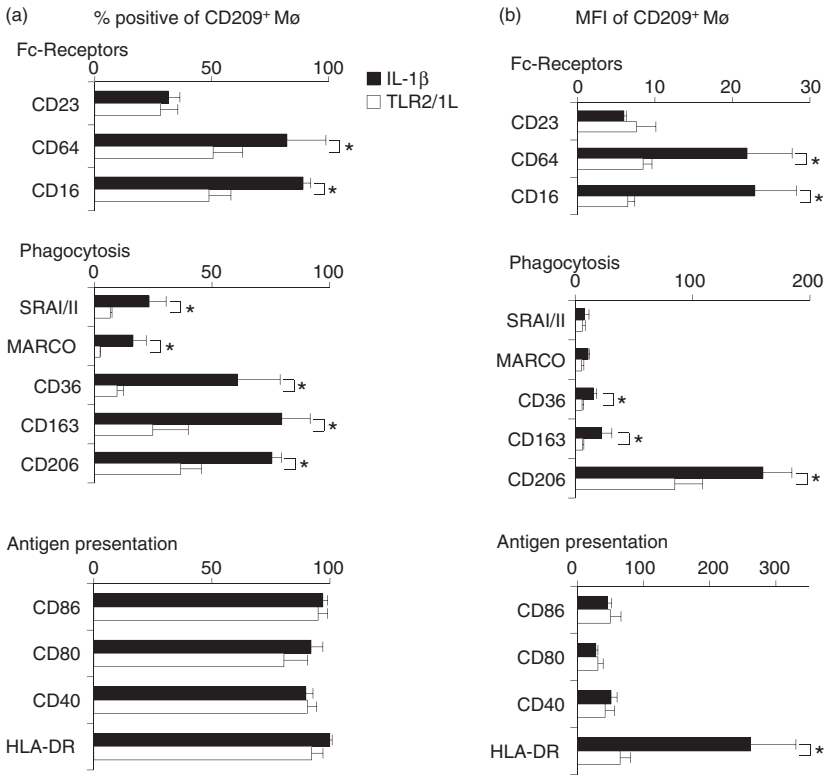

Figure 2. Expression of Fc-receptors, markers of phagocytosis and antigen presentation molecules on interleukin-1β (IL-1β) versus Toll-like receptor (TLR) 2/1L-induced CD209$^+$ macrophages (MΦ). Human peripheral monocytes were stimulated with IL-1β (closed bars) or TLR2/1L (open bars) for 2 days. The expression of cell surface markers on the CD209$^+$ MΦ was then analysed by flow cytometry. (a) The % marker positive cells and (b) the relative mean fluorescence intensities (MFI) for the markers are indicated for the CD209$^+$ MΦ. Data are shown as mean ± SEM; $n = 6$. Statistical significance was calculated by two-tailed Student's *t*-test.

given that these cells represent an MΦ cell type with moderate phagocytic function. In these experiments, we gated on the CD209$^+$ cells to identify MΦ. The MΦ induced by IL-1β demonstrated potent pinocytotic activity, as shown by uptake of Lucifer yellow (Fig. 3a). Even though IL-1β-induced MΦ showed the highest MFI for Lucifer yellow uptake (1·4-fold higher than the TLR2/1L-induced MΦ), the percentage of positive cells was equivalent for the different MΦ. To test for phagocytosis activity, we examined the uptake of oxLDL and mycobacteria BCG. The MFI for the uptake of oxLDL was highest in IL-1β-induced MΦ (1·7-fold higher than TLR2/1L induced MΦ) but no difference in the percentage positive cells was observed (Fig. 3b). Finally, the uptake of live mycobacteria, BCG, showed the highest MFI for the IL-1β-induced MΦ (1·7-fold higher than the TLR2/1L-induced MΦ and equivalent to IL-15 MΦ) but no significant difference in the percentage positive cells (Fig. 3c). To evaluate the ability of the cytokine-derived MΦ to bind particles or mycobacteria before uptake, MΦ were incubated as for the phagocytosis assays but at 4° instead of 37°.[1,11] The binding capacity did not vary between the different MΦ (see Supplementary material, Fig. S3). Therefore, our data suggest that the IL-1β-induced MΦ display the phenotypic and functional characteristics of MΦ with both pinocytic and phagocytic activity.

IL-1β-induced MΦ are more potent antigen-presenting cells than TLR2/1L-induced MΦ

Given that IL-1β-induced MΦ express higher levels of the antigen-presenting molecule HLA-DR, compared with TLR2/1L-induced MΦ, we tested differentiated MΦ for their potential to process and present antigen, by immunomagnetic selection of CD209$^+$ cells. For these experiments, we used the CD4$^+$, MHC class II-restricted T-cell clone, which recognizes an epitope from the *M. leprae* 10 000 molecular weight GroES protein.[12] As a control we used GM-CSF-induced CD1b$^+$ DC, enriched by immunomagnetic selection, known to be potent antigen-presenting cells for the GroES protein.[1] Using the GroES protein, IL-1β-derived MΦ induced up to 4·3-fold higher T-cell proliferation than the TLR2/1L-induced MΦ. In addition, IL-1β-derived MΦ, at the concentrations of 2×10^4 and 4×10^4 cells/well, induced significantly higher IFN-γ production, approximately twofold higher than the TLR2/1L-derived MΦ, but less than GM-CSF-derived DC (Fig. 4). Together these data suggest that the

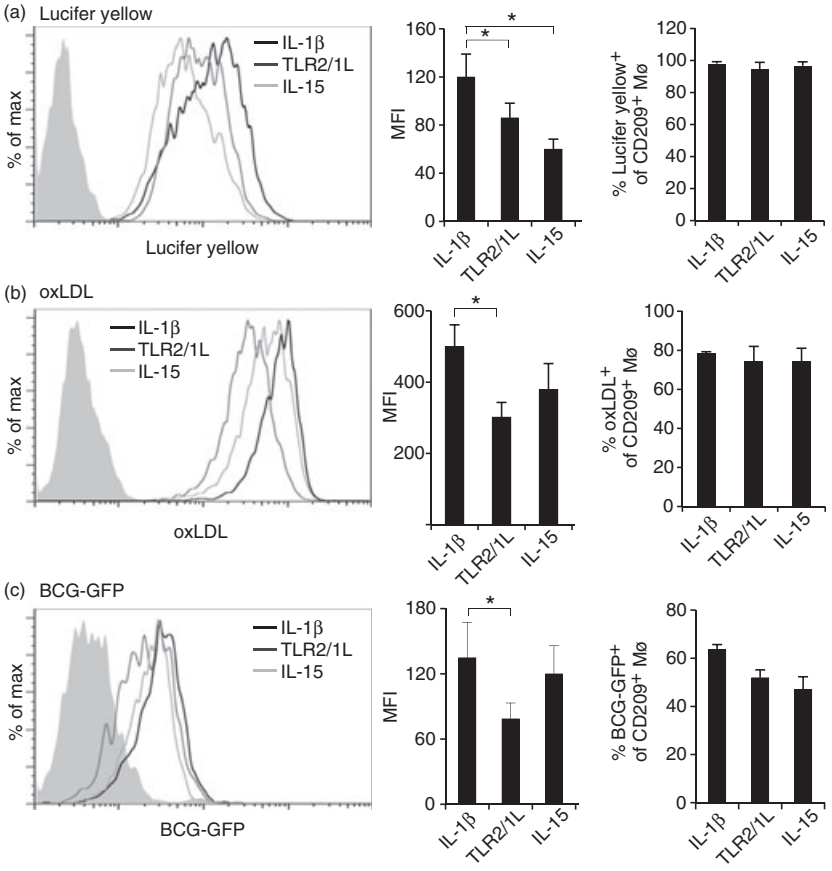

Figure 3. Interleukin-1β (IL-1β) -induced CD209$^+$ macrophages (MΦ) are potent phagocytic cells. Human peripheral monocytes were stimulated with IL-1β, Toll-like receptor (TLR) 2/1L or IL-15. After day 2 their phagocytic activity was measured by flow cytometry, showing specific uptake of (a) Lucifer yellow, (b) oxidized low-density lipoprotein (oxLDL) and (c) bacillus Calmette–Guérin-green fluorescent protein (BCG-GFP). A representative histogram and relative mean fluorescence intensities (MFI) are shown each gated on the marker positive CD209$^+$ MΦ population. Data are shown as mean fluorescence intensities and percentage positive indicated as mean $\pm$ SEM, $n = 7$. Statistical significance was calculated by two-tailed Student's *t*-test.

IL-1β-induced MΦ are antigen-presenting cells of intermediate potency, based on enhanced MHC II-restricted antigen-processing and presentation, compared with the TLR2/1L-induced MΦ, but diminished T-cell activation compared with the GM-CSF-derived DC.

Discussion

The innate immune programmes that regulate phagocytosis and antigen presentation are believed to be distinct and independent, and characterize MΦ and DC, respectively. By comparing the role of IL-1β with that of TLR2/1L in innate immune programming, we found that in addition to inducing monocytes to differentiate into CD1b$^+$ DC,[3] IL-1β also triggers monocytes to rapidly differentiate into CD209$^+$ MΦ. The IL-1β-derived CD209$^+$ MΦ showed phagocytic activity similar to TLR2/1L- and IL-15-derived MΦ, including the ability to take up live mycobacteria. In addition, IL-1β-derived CD209$^+$ MΦ were more efficient at processing and presenting mycobacterial antigen to MHC class II-restricted CD4$^+$ T cells, albeit not as efficient as GM-CSF-derived DC. Although IL-1β and TLR2/1L activate cells via MyD88, they do not trigger identical signalling pathways.[5] Therefore, the ability of IL-1β to trigger the differentiation of a functionally distinct subset of CD209$^+$ MΦ provides a distinct mechanism by which the innate immune system instructs the adaptive immune response against microbial pathogens. The identification that IL-1β by itself induces MΦ with enhanced antigen-presenting function is a first step in understanding the contribution of this pathway in the context of a complex mixture of immune stimuli.

A key finding of the present study was that IL-1β triggered the differentiation of monocytes into CD209$^+$ MΦ with both classical phagocytic activity and the ability to efficiently process and present antigen via MHC class II to T cells. Hence, IL-1β-derived CD209$^+$ MΦ are part of a family of MΦ with antigen-presenting function, including those derived by priming with IFN-γ and stimulating with either lipopolysaccharide alone or lipopolysaccharide plus immune complexes.[13,14] The combined phagocytosis and antigen-presenting function of the IL-1β-induced CD209$^+$ MΦ provides the innate immune system with the ability to simultaneously take up microbial pathogens and efficiently present them to T cells. In this context, the ability of IL-1β to also induce CD1b$^+$ DC,[3] which can present non-classical MHC antigens to T cells, provides a parallel pathway by which the innate immune system instructs the adaptive immune response.

The ability of IL-1β-derived CD209$^+$ MΦ to activate T cells that produce IFN-γ is also relevant for host defence against mycobacterial infection. IFN-γ is prominent in

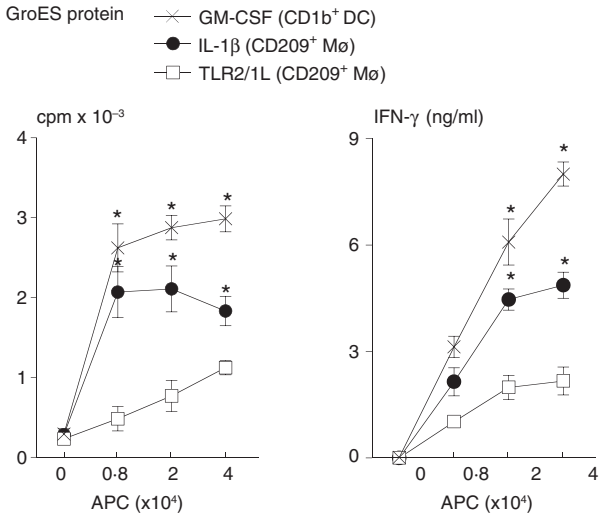

Figure 4. Interleukin-1β (IL-1β) -induced CD209$^+$ macrophages (MΦ) are able to process and present antigen. IL-1β and Toll-like receptor (TLR) 2/1L induced, magnetic-activated cell-sorted CD209$^+$ MΦ were compared to granulocyte–macrophage colony-stimulating factor (GM-CSF) -induced CD1b-sorted dendritic cells (DC) for the ability to present antigen to an MHCII-restricted T-cell clone (D103.5), which recognizes a peptide from the *Mycobacterium leprae* 10 000 molecular weight GroES protein. T-cell proliferation was measured using radioactive thymidine incorporation and interferon-γ (IFN-γ) production was measured by ELISA. Data are shown as mean of triplicate wells for one out of three independent experiments, $\pm$ SEM. Statistical significance was calculated by two-tailed Student's *t*-test.

the skin lesions of patients with the self-limited tuberculoid form versus the disseminated lepromatous form of leprosy,[15,16] and the administration of IFN-γ to patients with lepromatous leprosy transiently reduces the number of bacilli in lesions.[17] Levels of IFN-γ are also increased in tuberculosis pleuritis, a self-curing form of tuberculosis.[18] Although previous studies indicated that IFN-γ does not activate human MΦ to kill intracellular *M. tuberculosis*, recently it was shown that IFN-γ activates the vitamin D pathway in human MΦ resulting in a significant antimicrobial activity.[19]

The expression of CD209 on IL-1β-derived MΦ with both phagocytic and antigen-presenting function is probably relevant for the role of these cells in mycobacterial infection. Initially, CD209 was found to be expressed on DC *in vitro*, derived in the presence of GM-CSF and IL-4,[20] shown to be induced by IL-4.[21] However, it was subsequently reported that CD209 was not present on DC *in vivo*, but instead was a marker for tissue MΦ,[1] particularly specialized MΦ localized in the lymph node and at sites of inflammation.[22–25] The expression of CD209 on MΦ derived from monocytes via TLR activation[1] or by culture with various cytokines including IL-15,[1,11] is required for the ability of these MΦ to bind and phagocytose mycobacteria, including *M. leprae*.[26] CD209$^+$ MΦ are also prominent at the site of disease in tuberculosis[27] and leprosy.[1] Previous studies report that a single nucleotide polymorphism (−336A/G) in the CD209 promoter diminishes expression and is associated with human susceptibility to tuberculosis.[28,29] In particular, CD209 expression is protective against cavitary tuberculosis.[28] CD209 expression, and hence CD209$^+$ MΦ, has also been implicated in host defence against HIV and Dengue virus infection.[30,31] In our work we used CD209 as macrophage marker, however its involvement in binding and subsequent uptake of mycobacteria has been previously shown.[1]

A role for IL-1β in host defence against mycobacterial infection has been suggested by the demonstration that in human leprosy, IL-1β is more strongly expressed in lesions from patients with the self-limited tuberculoid form versus the disseminated lepromatous form of leprosy[15] and participates at the site of disease in tuberculosis.[32] A high IL-1β/low IL-1RA-producing haplotype has been associated with pleural tuberculosis, a form of the disease that generally resolves without chemotherapy.[33] Furthermore, mouse studies suggest an important role of IL-1β in tuberculosis: IL-1α and IL-1β double-knockout mice and IL-1R type I-deficient mice (which do not respond to IL-1) display an increased mycobacterial growth and also defective granuloma formation after infection with *M. tuberculosis*.[34,35] In humans, IL-β has been shown to contribute to monocyte/MΦ antimicrobial activity against *M. tuberculosis* via induction of antimicrobial peptides[36] as well as via induction of caspase 3.[37] Together, these data suggest the possibility of targeting the IL-1β MΦ pathway as an adjunct to therapy for advanced or drug-resistant mycobacterial infection, to induce an antimicrobial response as well as CD209$^+$ MΦ with phagocytic and antigen-presenting function.

Authorship

RLM, SRK and MS designed the research and wrote the paper. MS performed most of the experiments. MF and PAS performed the antigen presentation experiments. DMV was involved in the cell differentiation and flow cytometry experiments. DJL, DM and PTL helped with interpreting the results. All authors reviewed and provided input toward the final paper.

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Disclosures

The authors declare no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. IL-1p induces CD209 in a dose-dependent manner. Purified human monocytes were activated with interleukin-1β (IL-1β) for 48 hr and analysed by flow cytometry for the expression of CD209 (mean fluorescence intensity; MFI). CD209 expression is shown as mean percentage positive, $\pm$ SEM. $n = 3$.

Figure S2. IL-1p induces $CD1b^+$ dendritic cells (DC). Human peripheral monocytes were stimulated with interleukin-1β (IL-1β) or left untreated. (a) After day 2 and 3 cells were analysed by flow cytometry for the expression of CD1b. (a) A representative double labelling for CD1b and CD209 is shown. (b) The percentage of CD1b-positive cells is shown for untreated and IL-1β-treated monocytes after days 2 and 3 as mean $\pm$ SEM; $n = 5$. Statistical significance was calculated by two-tailed Student's *t*-test.

Figure S3. Binding capacity of macrophages (MΦ). Human peripheral monocytes were stimulated with interleukin-1β (IL-1β), Toll-like receptor (TLR) 2/1L or IL-15. After day 2 their binding capacity was measured by flow cytometry, showing binding at 4° of (a) Lucifer yellow, (b) oxidized low-density lipoprotein (oxLDL) and (c) bacillus Calmette–Guérin-green fluorescent protein (BCG-GFP). A representative histogram gated on the $CD209^+$ MΦ population is shown.

