IAI
Journals.ASM.org

Global Effect of Interleukin-10 on the Transcriptional Profile Induced by *Neisseria meningitidis* in Human Monocytes

Unni Gopinathan,[a,c] Reidun Øvstebø,[a] Ole Kristoffer Olstad,[a] Berit Brusletto,[a] Hans Christian Dalsbotten Aass,[a] Peter Kierulf,[a,c] Petter Brandtzaeg,[a,b,c] and Jens Petter Berg[a,c]

Blood Cell Research Unit, Section for Research, Department of Medical Biochemistry, Oslo University Hospital,[a] Department of Pediatrics, Oslo University Hospital,[b] and Institute of Clinical Medicine, Faculty of Medicine, University of Oslo,[c] Oslo, Norway

In meningococcal septic shock, the dominant inducer of inflammation is lipopolysaccharide (LPS) in the outer membrane of *Neisseria meningitidis*, while interleukin-10 (IL-10) is the principal anti-inflammatory cytokine. We have used microarrays and Ingenuity Pathway Analysis to study the global effects of IL-10 on gene expression induced by *N. meningitidis*, after exposure of human monocytes ($n = 5$) for 3 h to *N. meningitidis* (10^6 cells/ml), recombinant human IL-10 (rhIL-10) (25 ng/ml), and *N. meningitidis* combined with rhIL-10. *N. meningitidis* and IL-10 differentially expressed 3,579 and 648 genes, respectively. IL-10 downregulated 125 genes which were upregulated by *N. meningitidis*, including NLRP3, the key molecule of the NLRP3 inflammasome. IL-10 also upregulated 270 genes which were downregulated by *N. meningitidis*, including members of the leukocyte immunuglobulin-like receptor (LIR) family. Fifty-three genes revealed a synergistically increased expression when *N. meningitidis* and IL-10 were combined. AIM2 (the principal molecule of the AIM2 inflammasome) was among these genes (fold change [FC], 18.3 versus 7.4 and 9.4 after stimulation by *N. meningitidis* and IL-10, respectively). We detected reduced concentrations (92% to 40%) of six cytokines (IL-1b, IL-6, IL-8, tumor necrosis factor alpha [TNF-α], macrophage inflammatory protein alpha [MIP-α], MIP-β) in the presence of IL-10, compared with concentrations with stimulation by *N. meningitidis* alone. Our data analysis of the effects of IL-10 on gene expression induced by *N. meningitidis* suggests that high plasma levels of IL-10 in meningococcal septic shock plasma may have a profound effect on a variety of functions and cellular processes in human monocytes, including cell-to-cell signaling, cellular movement, cellular development, antigen presentation, and cell death.

Neisseria meningitidis is the cause of epidemic meningitis and fulminant septicemia with a constant high case fatality rate (49). The compartmentalization of the infection to the cerebrospinal fluid causes the clinical picture of distinct meningitis, while systemic infection may cause mild meningococcemia or fulminant septicemia (49, 52). The clinical manifestations are associated with the circulating level of lipopolysaccharide (LPS) and DNA derived from *N. meningitidis* and the inflammatory response in the cardiovascular system or the subarachnoid space (8). A dose-response relation exists between the plasma level of LPS and fatal septic shock (5, 6). The complex reaction of the innate immune system to the intruding meningococci is reflected in the profile of inflammatory mediators elicited during infection as measured in plasma and cerebrospinal fluid (5).

We have been particularly interested in the behavior of human monocytes as phagocytes in the circulation and as cellular inducers of coagulopathy when they encounter *N. meningitidis* in patients with systemic meningococcal disease. Human monocytes are highly responsive to meningococci. *N. meningitidis* and purified LPS from *N. meningitidis* induce an altered expression of more than 4,600 genes (43). Still, *in vitro* studies combining plasma from patients with fulminant septicemia with normal human donor monocytes suggest that the monocytes may not be activated in a shock plasma sample despite concurrent high levels of bioactive LPS (7). Interleukin-10 (IL-10) was subsequently identified as the single most important plasma factor suppressing monocyte ability to produce proinflammatory cytokines and induce procoagulant activity (7). IL-10 is a major anti-inflammatory regulator of myeloid cells activated by bacteria or LPS (12). The suppressing effect of IL-10 on human monocytes has previously been associated with an altered expression of comparatively few genes (1, 24, 54). *In vitro* studies suggest that IL-10 is released with slower kinetics than most of the proinflammatory cytokines (5, 20). However, patients with fulminant meningococcal sepsis have a 10^3 to 10^4 increase in IL-10 concentrations in plasma when they reach the hospital 8 to 24 h after the first symptoms are recognized (5, 7). A dose-response relation appears to exist between the levels of LPS and IL-10 in meningococcal shock plasma (5). The data suggest that IL-10 increases in parallel with the intravascular growth of the meningococci, as has been shown for a large number of proinflammatory cytokines and their inhibitors (5).

The primary aim of this study was to elucidate the global effect of IL-10 on gene expression in human monocytes stimulated *in vitro* by a reference strain of *N. meningitidis*, compared with monocytes stimulated *in vitro* by *N. meningitidis* or IL-10 separately. Second, using Ingenuity Pathway Analysis (IPA), we aimed at identifying key molecules and functional groups of genes affected by IL-10 in the gene expression profile induced by *N. meningitidis* in human monocytes. Finally, we quantified clinically important proinflammatory cytokines in the supernatant from these

Received 19 April 2012 Returned for modification 24 May 2012
Accepted 30 August 2012
Published ahead of print 10 September 2012
Editor: J. N. Weiser
Address correspondence to Unni Gopinathan, unni.gnathan@gmail.com.
Supplemental material for this article may be found at http://iai.asm.org/.
Copyright © 2012, American Society for Microbiology. All Rights Reserved.
doi:10.1128/IAI.00386-12

monocytes, evaluating the relation between altered gene expression and protein secretion to the culture medium.

MATERIALS AND METHODS

Equipment and reagents. All reagents and solutions were analyzed for the presence of LPS using the *Limulus* amebocyte lysate (LAL) assay (Pyrochrome). The lower detection limit was 0.16 endotoxin units (EU)/ml.

Pooled human normal plasma. Heparinized whole blood was collected from consenting, healthy donors ($n = 10$) and immediately centrifuged (1,400 $\times$ *g*, 10 min, 20°C). Plasma was pipetted off, mixed, aliquoted, and stored at -70°C.

The heparin concentration in the plasma is 30 IU/ml. Previous studies have shown that heparin may inhibit the production of cytokines from human monocytes (17, 19).

To rule out a possible inhibitory effect of the heparin plasma on the monocytes' ability to synthesize and secrete tumor necrosis factor alpha (TNF-α), we compared TNF production from monocytes cultured with meningococci in 50% heparin plasma with that of those in 50% serum. The TNF-α median level in 50% serum was 80% (range, 16 to 86%; $n = 3$) compared with that in heparin plasma. From these results, the heparin plasma did not appear to induce an inhibitory effect on human monocytes stimulated with *N. meningitidis*.

FBS. Fetal bovine serum (FBS) (Biowhittaker) was tested to contain $<$1 EU/ml of LPS and was heat inactivated to inactivate inhibitory serum proteases and complement proteins.

N. meningitidis. *N. meningitidis* strain 44/76 (serogroup B) was isolated from a culture of blood from a Norwegian patient with lethal meningococcal infection. The bacteria were serologically characterized, cultured, harvested, and heat inactivated at the Norwegian Institute of Public Health as previously described (43).

Quantification of the number of bacteria by qPCR (*N. meningitidis* DNA quantification). Ten microliters of solution with heat-inactivated *N. meningitidis* was added to 190 μl of pooled, normal plasma from heparinized blood and subjected to robotized isolation of *N. meningitidis* DNA (MagNA Pure LC robot; Roche). Quantification of *N. meningitidis* DNA was performed using quantitative PCR (qPCR) as previously described (42), except for the sequence-specific hybridization probes (0.3 mol per reaction) 5′-AGGATACG AATGTGCAGCTGAC-FL and 5′-LC Red640-GTGGCAATGTAGTACGAA CTGTTGC-PH (TIB Molbiol), and the Light Cycler Fast Start DNA master hybridization probe mix was used as the detection system.

Monocyte target assay. Elutriation-purified, cryopreserved human monocytes ($>$90% purity) (31) from consenting, healthy donors ($n = 5$) were thawed and resuspended in 5% fetal calf serum (FCS)-RPMI 1640 (vol/vol) containing 2% (vol/vol) penicillin-streptomycin and seeded (1.5×10^6 monocytes suspended in 500 μl 5% FCS-RPMI/well) in microtiter plates (Costar 3524). A 500-μl portion of 5% FCS-RPMI containing 2.0×10^6 cells/ml *N. meningitidis*, 50 ng/ml recombinant human IL-10 (rhIL-10), and 2.0×10^6 cells/ml *N. meningitidis* in combination with 50 ng/ml rhIL-10 or vehicle (control) was added to duplicate wells together with 1,000 μl pooled normal human plasma (total volume, 2,000 μl/well). The final concentrations of the constituents in the respective wells were 7.5×10^5/ml monocytes, 10^6 cells/ml *N. meningitidis*, and 25 ng/ml rhIL-10. In the wells containing both rhIL-10 and *N. meningitidis*, rhIL-10 was added to the wells after adding *N. meningitidis*, in order to avoid preincubation with IL-10. Plates were sealed off and incubated for 0 and 3 h (37°C, 5% CO_2). A 3-h incubation was chosen, since pilot experiments and our previous study had indicated that 3 h of incubation was adequate for studying differential expression of genes in human monocytes stimulated by *N. meningitidis* (43). The selected concentration of IL-10 was determined by dose-response studies of its ability to inhibit TNF-α production in human monocytes stimulated by *N. meningitidis* (data not shown). This concentration was also in the range of previously measured concentrations of IL-10 in meningococcal septic shock plasma (7, 27, 47).

Each plate was then centrifuged (47 $\times$ *g*, 5 min, room temperature), and the supernatant was gently removed, aliquoted, and stored at -70°C until assayed for released proteins by the Luminex system (see below). Subsequently, the cells were harvested and stored in 700 μl Qiazol lysis buffer (supplied with the miRNeasy minikit from Qiagen) at -70°C until subjected to total RNA isolation (see below).

Viability of the monocytes. The cell viability has previously been analyzed using a FACSVantage DiVa flow cytometer with 488 nm excitation of annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI), detected through 530/22 and 630/22 filters, respectively, before cryopreservation. We have previously shown, based on counting the number of cells (Advia 60 hematology system) and detecting viability (annexin V and PI), that the viability and number of monocytes seeded and stimulated with *N. meningitidis* (10^6 cells) or vehicle for 3 h essentially remain the same before and after incubation (43).

RNA preparation. Total RNA was extracted using the miRNeasy minikit (Qiagen) according to the manufacturer's instructions. The isolated total RNA was quantified (NanoDrop spectrophotometer) and quality controlled using the RNA 6000 Nano assay (Agilent BioAnalyzer 2100 system), giving RNA integrity number values ranging from 6.9 to 8.8.

Microarray analyses and statistical analysis of data. Microarray analyses were performed using the Affymetrix GeneChip human gene 1.0 ST arrays (Affymetrix), which contain approximately 28,000 gene transcripts. Total RNA (150 ng) was subjected to the GeneChip HT one-cycle cDNA synthesis kit and GeneChip HT IVT labeling kit, following the manufacturer's protocol for whole-genome gene expression analysis (Affymetrix). Biotinylated and fragmented single-stranded cDNAs were hybridized to the GeneChips. The arrays were washed and stained using an FS-450 fluidics station (Affymetrix). Signal intensities were detected by a Hewlett Packard 3000 7G gene array scanner. The scanned images were processed using the AGCC (Affymetrix GeneChip Command Console) software, and the CEL files were imported into Partek Genomics Suite software. The robust multichip analysis (RMA) algorithm was applied for generation of signal values and normalization.

Gene transcripts with maximal signal values of less than 32 across all arrays were removed to filter for low and nonexpressed genes, reducing the number of gene transcripts to 22,432. For expression comparisons of different groups, profiles were compared using a 2-way analysis of variance (ANOVA) model. The results were expressed as fold change (FC). Gene lists were generated with the criteria of a *P* value of $<$0.01 and an FC of $\geq$1.5. We decided upon a fold change at this level of significance in order to capture a broader range of *N. meningitidis*-stimulated genes affected by IL-10.

Ingenuity Pathway Analysis (IPA). The functional analysis and gene networks were generated through the use of Ingenuity Pathway Analysis. Data sets containing gene identifiers and corresponding expression values were uploaded into the application. Each identifier was mapped to its corresponding object in the Ingenuity Knowledge Base. Fisher's exact test was performed to calculate a *P* value determining the probability that each biological function assigned to the data set was due to chance alone. A cutoff at $P < 0.01$ and FC ≥ 1.5 was set to identify molecules whose expression was significantly differentially regulated. Venn analysis was used to identify the overlap of probe set IDs differentially expressed in monocytes stimulated by *N. meningitidis* (10^6 cells/ml), IL-10 (25 ng/ml), and *N. meningitidis* (10^6 cells/ml) combined with IL-10 (25 ng/ml), compared with unstimulated monocytes (control).

The significantly expressed genes, called network eligible molecules, were overlaid onto a global molecular network developed from information contained in the Ingenuity Knowledge Base. Gene networks were generated based on the existing scientific information in the Ingenuity Knowledge Base on the molecular relationships between the network eligible molecules. IPA computes a score for each network according to the fit of the genes in the data set, in order to identify the networks which are most highly expressed. This score is generated using a *P* value calculation

determined by a right-tailed Fisher's exact test, and it is displayed as the negative log of that *P* value. This score indicates the likelihood that the fit of the set of focus genes in a network could be explained by chance alone. A score of 2 indicates that there is a 10^{-2} chance that the focus genes are together in a network by chance.

Reverse transcription-PCR. The differential gene expression data were validated for selected transcripts using the TaqMan gene expression assays and the Applied Biosystems ViiA7 real-time PCR system. Pooled total RNA (100 ng, 20 ng from each of the 5 donors) from human monocytes induced by *N. meningitidis*, rhIL-10, *N. meningitidis* in combination with rhIL-10, or vehicle was reverse transcribed using Omniscript (Qiagen Ltd.). cDNA (9 μl), diluted 1:10 in H_2O, and 1 μl of IL-1β-Hs 01555410_m1, TNFα-Hs 99999043_m1, CCL3-Hs 00234142_m1, CXCL10-Hs00171042_m1, SLAMF1-Hs 00900290_m1, AIM2-Hs 00915710_m1, CASP5-Hs 00362078_m1, NLRP3-Hs 00918082_m1, EDN1-Hs 00174961_m1, F3-Hs 01076029_m1, RIPK2-Hs 01572686_m1, B2M-Hs 00187842_m1, TMBIM4-Hs 00187842_m1, or PPIB-Hs 00168719_m1 were added to 10 μl TaqMan universal PCR master mix (Applied Biosystems). The relative changes of each transcript, using a geometric mean of results for TMBIM4-Hs 00211390_m1, PPIB-Hs 00168719_m1, and β_2M-Hs 00984230_m1 as endogenous controls (45), were calculated using the software ViiA7RUO software and the $\Delta\Delta C_T$ method (C_T, threshold cycle) (28). The results are expressed as relative quantities, e.g., the amount of target (*N. meningitidis*, rhIL-10, *N. meningitidis* in combination with rhIL-10 in incubated monocytes) normalized to the geometric mean for the endogenous references and relative to the quantity of monocytes incubated with vehicle (control).

Quantification of protein levels in supernatants. Supernatants, harvested from human monocytes incubated with *N. meningitidis*, rhIL-10, *N. meningitidis* in combination with rhIL-10, or vehicle, were analyzed using a microsphere-based multiplexing bioassay system with Xmap technology (Luminex Corporation). The culture supernatants were examined for 30 cytokines (epidermal growth factor [EGF], eotaxin, fractalkine, granulocyte colony-stimulating factor [G-CSF], granulocyte-macrophage CSF [GM-CSF], gamma interferon [IFN-γ], IL-1α, IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 [p40, free form], IL-12 [p70], IL-13, IL-15, IL-17, CXCL10 [IP10], monocyte chemoattractant protein 1 [MCP-1], macrophage inflammatory protein-α [MIP-α], MIP-1β, RANTES, soluble CD40L, transforming growth factor alpha [TGF-α], TNF-α, and vascular endothelial growth factor [VEGF]) using the Human Cytokine/Chemokine Lincoplex kit (Millipore). All supernatants from each experiment ($n = 5$) were run in parallel. The analyses were performed according to the protocol of the manufacturer, who reported interassay variation of 3.7 to 17.2% (coefficient of variation [CV]) and intra-assay variation of 4.6 to 13.8% (CV).

Statistical analysis. The measurements from the culture supernatants are given as means and standard errors of the means. Statistical analysis comparing means in culture supernatants from *N. meningitidis* and *N. meningitidis* in combination with IL-10 was performed with paired *t* tests in SPSS 14.0. A *P* value of 0.01 was assigned after Bonferroni correction for multiple samples.

Pearson's correlation test was used to calculate the correlation between fold changes in Affymetrix and reverse transcription-PCR (RT-PCR).

RESULTS AND DISCUSSION

Descriptive analysis of the differentially expressed genes. The present study revealed a total of 3,579 differentially expressed genes (872 upregulated and 2,707 downregulated) in human monocytes when incubated for 3 h with *N. meningitidis* (10^6 cells/ml) compared with controls (cutoff FC, ≥1.5, $P < 0.01$) (Table 1). The expression pattern resembled the results from our previous study (43), although we have used different criteria for cutoff for the purpose of identifying a broader range of genes affected by IL-10. When the monocytes were stimulated by *N. meningitidis* in combination with IL-10, 2,316 genes (870 upregulated and 1,446 downregulated) were differentially expressed compared with controls. Previous studies on IL-10 gene expression profiles in LPS-stimulated human monocytes and macrophages have largely focused on the inhibitory effect of IL-10 (1, 24, 54). In our study, 555 of 872 genes induced by *N. meningitidis* remained significantly expressed (fold change, ≥1.5; $P < 0.01$) when IL-10 was present. A total of 317 genes were thus no longer significantly expressed due to the presence of IL-10. IL-10 also reduced the number of genes significantly downregulated by *N. meningitidis.* Of the 2,707 downregulated genes, only 1,244 genes remained significantly regulated in the presence of IL-10 (Table 1). Considering that we were measuring expression levels as early as after 3 h of stimulation, it seems that IL-10 imposes a rapid and potent effect on a large portion of the genes differentially expressed by *N. meningitidis.*

TABLE 1 Numbers of differentially expressed genes in human monocytes[a]

Gene type	No. of genes in monocytes incubated with[b]:		
	N. meningitidis	IL-10	*N. meningitidis* plus IL-10
Differentially expressed	3,579	648 (263)	2,316 (1,799)
Upregulated	872	297 (69)	870 (555) [189]
Downregulated	2,707	351 (194)	1,446 (1,244) [163]
Eligible for Ingenuity Pathway Analysis	2,994	541	1,951

[a] Numbers of differentially expressed genes in human monocytes incubated with *N. meningitidis*, IL-10, and both for 3 h, analyzed versus unstimulated monocytes.
[b] Criteria for cutoff: FC ≥ |1.5|, $P < 0.01$. Parentheses show the number of genes in the expression profile induced by incubating with *N. meningitidis* alone. Square brackets show the number of genes only expressed in the expression profile induced by incubating with both *N. meningitidis* and IL-10.

Previous expression profiling studies using IL-10 (1, 24, 54) have to a limited extent studied whether IL-10 and LPS together induce a separate set of genes compared with those induced by their individual effects on monocytes and macrophages (1, 24, 54). We identified 352 genes (189 upregulated and 163 downregulated) to be significantly regulated only in monocytes stimulated by the combination of IL-10 and *N. meningitidis* and not in monocytes stimulated by *N. meningitidis* or IL-10 alone (Table 1), all compared with controls. The potential role of this group of genes and their gene products in the pathophysiology of meningococcal septic shock has, to our knowledge, not previously been addressed.

We used IPA to allocate genes differentially expressed by *N. meningitidis* to related molecular and cellular functions. The presence of IL-10 reduced the number of significantly regulated genes associated with the different functional groups induced by *N. meningitidis*, showing IL-10's ability to exert a global effect on monocyte function (Table 2). The wide range of functions affected by IL-10 may indicate why the inhibitory and anti-inflammatory effect of IL-10 has been difficult to use therapeutically (2, 41).

When IL-10 was incubated with human monocytes alone, it induced the differential expression of 648 genes (297 upregulated and 351 downregulated) compared with controls (Table 1). Williams et al. identified 19 genes induced by IL-10 with FCs of ≥3.0 and concluded that IL-10 induced a limited number of genes in human monocytes (54). Lang et al. identified 37 genes to be upregulated and 22 genes to be downregulated by IL-10 (FC ≥ |2.0|)

TABLE 2 Numbers of differentially expressed genes involved in biological functions[a]

	No. of genes in monocytes incubated with[b]:		
Function of differentially expressed genes	*N. meningitidis*	IL-10	*N. meningitidis* plus IL-10
Cell death	120	52 (31)	99 (78)
Cellular development	162	58 (33)	144 (110)
Cellular movement	139	53 (30)	120 (86)
Cell-to-cell signaling and interaction	166	85 (44)	155 (105)
Cellular growth and proliferation	155	57 (30)	147 (106)

[a] Number of differentially expressed genes involved in biological functions in human monocytes incubated with *N. meningitidis*, IL-10, and both for 3 h, analyzed versus unstimulated monocytes. Functional analysis performed by Ingenuity Pathway Analysis.
[b] Criteria for cutoff: FC ≥ |1.5|, $P < 0.01$. The parentheses contain the number of genes found in the expression profile induced by *N. meningitidis* alone.

in bone marrow-derived macrophages from mice after 3 h (24), while Antoniv et al. upregulated the expression of 273 genes and downregulated the expression of 71 genes by an FC of ≥|2.0| after 6 h of IL-10 incubation (1). Comparing our study with these studies may be difficult due to different cell types and species, experimental conditions, different microarray technologies, and the cutoff criteria. The larger number of genes we have identified to be induced by IL-10 can partially be explained by a lower cutoff value (FC ≥ 1.5) than in related studies. However, when we increased the cutoff to an FC of ≥|2.0|, we still observed upregulation of 82 and downregulation of 62 genes ($P < 0.01$) by IL-10. Our results suggest that IL-10 alone influences the expression of a larger number of genes in human monocytes at 3 h than previously reported.

In order to further analyze the specific effects of IL-10 in the presence of *N. meningitidis*, we will in the following sections present four groups of genes: (i) genes upregulated by *N. meningitidis* which were downregulated by IL-10, (ii) genes downregulated by *N. meningitidis* which were upregulated by IL-10, (iii) genes synergistically upregulated by *N. meningitidis* in combination with IL-10, and (iv) genes synergistically downregulated by *N. meningitidis* in combination with IL-10. We have used IPA to examine the functional categories to which these genes belong and generated networks showing the molecular relationships between the genes in these groups.

Identification of genes upregulated by *N. meningitidis* which were downregulated by interleukin-10. We identified 125 genes which were upregulated by *N. meningitidis* and inhibited in the presence of IL-10 with an FC of ≤−1.5 ($P < 0.01$) (see Table S1 and the heatmap in Fig. S1 in the supplemental material). Lang et al. found in a mouse model that of 259 genes upregulated with an FC of ≥3.0 by LPS, 62 were repressed with an FC of ≥1.5 by IL-10 (24). Williams et al. also observed that IL-10 downregulated a large number of genes upregulated by LPS from *Salmonella enterica* serovar Typhimurium (54).

In general, our results reflected the findings of these studies. In our previous study of genes differentially expressed in human monocytes stimulated by *N. meningitidis* and LPS purified from *N. meningitidis*, we showed that 1,273 genes were unique for *N. meningitidis*, while 878 genes were unique for purified LPS. These results suggested that the presentation form of LPS may influence the gene expression pattern. Williams et al. used purified LPS from *Salmonella* Typhimurium, which is structurally and physicochemically different from membrane-bound LPS as it occurs in *N. meningitidis* (5, 46). Furthermore, previous studies have not systematically analyzed the range of functional groups to which the affected genes belong or the possible networks and interactions these genes may have. The molecular and cellular functions mostly associated with the genes inhibited by IL-10 were cellular development (14 genes), cell-to-cell signaling and interaction (18 genes), cellular movement (15 genes), antigen presentation (16 genes), and cellular function and maintenance (13 genes). We utilized IPA to generate gene networks displaying the molecular relationships between the genes inhibited by IL-10. The networks are generated based upon previously validated molecular relationships that have been entered into the Ingenuity Knowledge Base. However, we did not limit our results to those that have previously been reported to be relevant for human monocytes, in order to avoid losing information about possible relationships which have not been validated or entered into the database previously.

The analysis resulted in two networks for which IPA calculated the significant scores of 19 and 7 (Table 3; see Fig. S2 and Fig. S3 in the supplemental material), indicating that the relationships

TABLE 3 Network generation of genes upregulated by *N. meningitidis* and downregulated by IL-10[a]

Network	Molecules in network[b]	Score[c]	Top functions
1	AKT2, C5r, CD40LG, CEBPB, **CSF1, CSF2, CXCL2, CXCL9, CXCL11**, ICOS, **IFNG, IL-6**, IL-13, **IL12A, IL1A, IL23A**, IL23A, IL3RA, IRAK3, IRF1, IRF3, IRF4, IRF8, MAPK13, **MMP1**, RAF1, **RIPK2**, RPS6KA3, SFTPD, **SPP1, ST8SIA4**, STAT1, STAT4, TLR4, **TNF**	19	Respiratory disease, cellular development, hematological system development and function
2	AKT1, BCL3, C5, CCL2, CCL3, CCL4, CCL5, CD163, **CXCL3, F3**, FOXP3, ICOS, **IFNG**, IL-4, IL-8, IL-9, IL17A, IL1R1, IRF3, Klrb1c, LBP, MAPK14, MYD88, **NFIL3**, NFKBIA, **NLRP3, NRP1, PDE48**, PF4, **RGS1**, STAT1, TICAM1, TLR3, TLR4	7	Inflammatory response, cellular movement, hematological system development and function

[a] Gene networks were generated based on existing scientific information about the molecular relationships between genes and gene products in the Ingenuity Knowledge Base.
[b] Genes in bold are the focus molecules which were significantly upregulated by *N. meningitidis* and downregulated by IL-10.
[c] The score indicates the likelihood that the relationships in the network could be explained by chance alone. A score of 2 would indicate that there is a 10^{-2} probability that the focus molecules are in a network together by chance.

found in the gene networks were statistically significant (see Materials and Methods for further explanation). The most significant functions related to the genes of the first network were cellular development and hematological system development and function. The subcellular layout function in IPA showed that 13 of the 15 gene products in this network were located in the extracellular space. One gene located in the plasma membrane, RIPK2 (also known as RIP2, RICK, or CARDIACK), is an important effector molecule for the pattern recognition receptors NOD1 and NOD2 (39). RIPK2 has previously been implicated in TLR signaling in response to LPS. Studies have shown that RIPK2-deficient macrophages stimulated with LPS have reduced cytokine production and decreased NF-kappaB activation mediated by Toll-like receptor 4 (TLR4) (11) and that RIPK2 directly participates in the LPS/TLR4 signaling pathway (29). However, a later study found that the production of cytokines induced by LPS and other TLR ligands was independent of RIPK2 but that it was important for the cooperation between NOD2 and TLR signaling in the production of cytokines (44). We found RIPK2 to be upregulated by *N. meningitidis* (FC = 3.7) after 3 h of stimulation but reduced when IL-10 was present (FC = −2.6). Due to its possible role in TLR4 signaling and cytokine production, RIPK2 may be a potential target gene for the IL-10 anti-inflammatory response. To our knowledge, the interaction between IL-10 and RIPK2 has not previously been described in the literature.

The second most significant network was characterized by genes related to inflammatory response, cellular movement, and hematological system development and function. One of the most interesting discoveries in this network was the gene which encodes NLRP3, a protein belonging to a family of cytosolic pattern recognition receptors. Activation of NLRP3, in response to microbial ligands in the cytosol, results in the assembly of the NLRP3 inflammasome complex (50). This protein complex mediates caspase-1 activation, resulting in IL-1β synthesis (50). Caspase-1 is constitutively activated in human monocytes in the presence of the inflammasome assembled by NLRP3 and ASC (50). Recent data suggest that LPS-induced release of IL-1β in THP-1 cells and peripheral blood mononuclear cells relies on signaling through NLRP3 or apoptotic speck-like protein containing a caspase recruitment domain (ASC), since transfection of small interfering RNA (siRNA) targeting NLRP3 or ASC can inhibit the LPS-stimulated release of IL-1β in these cells (40). Furthermore, IL-1β induction in LPS-stimulated monocytes and macrophages has been shown to be caspase-1 dependent (32). In our network we found that NLRP3 was upregulated by *N. meningitidis* (FC = 3.5) but significantly inhibited in the presence of IL-10 combined with *N. meningitidis* (FC = −1.9). The discovery of NLRP3 among the significantly expressed network of genes stimulated by *N. meningitidis* and inhibited in the presence of IL-10 identifies it as a possible target for the IL-10 anti-inflammatory response. IL-10 may thus have a role in regulating inflammasome activation in response to pathogens.

In both networks, we found that IL-10 inhibited the expression of genes encoding cytokines and chemokines (CSF1, CSF2, TNF-α, IL-1α, IL-6, IL12A, IL23A, IFNG, CXCL2, CXCL9, CXCL12), in accordance with previous studies (48).

Identification of genes inhibited by *N. meningitidis* which were upregulated by IL-10. The inhibitory effect of IL-10 on LPS activation has received substantial attention (37, 38). It has previously been proposed that specific downstream mediators of the IL-10 anti-inflammatory response, such as genes encoding transcriptional factors, may be found among the genes upregulated by IL-10 in the presence of LPS (24).

We identified 270 genes inhibited by *N. meningitidis* that were significantly upregulated (FC ≥ 1.5) in the presence of IL-10 (see Table S2 and the heatmap in Fig. S4 in the supplemental material). When we compared these 270 genes with the 297 genes upregulated by IL-10 alone in human monocytes (Table 1) in a Venn diagram, we found only 44 genes to be in common. This is in agreement with the results of Lang et al., who showed that IL-10 upregulated a restricted number of genes in unstimulated macrophages from mice, whereas IL-10 upregulated a different set of genes in LPS-stimulated macrophages (24). The upregulation of a separate set of genes by IL-10 in monocytes stimulated with *N. meningitidis*, compared with upregulation in monocytes stimulated by IL-10 alone, shows that the IL-10 effect in monocytes may be "reprogrammed" in response to *N. meningitidis*. Genes related to cellular development (16 genes), antigen presentation (10 genes), cell-to-cell signaling and interaction (18 genes), cellular movement (10 genes), and cell death (16 genes) were the most significantly induced.

From the list of genes upregulated by IL-10, IPA generated three gene networks with the significant scores of 16, 16, and 12 (Table 4; see Fig. S5, Fig. S6, and Fig. S7 in the supplemental material). The top functions designated by IPA to these networks were cell death, cellular growth and proliferation, hematological system development and function, tissue morphology, inflammatory response, and cellular movement. When we organized the network according to subcellular layout, we identified five transcriptional regulators significantly upregulated in response to IL-10 stimulation. These were ARNTL2, BCL3, IRF5, PLAGL2, and VAV1. ARNTL2, PLAGL2, and VAV1 have so far been described in relation to malignancies (21, 23, 34) and diabetes (16).

IRF5 belongs to the interferon regulatory factor family (IRFs 1 to 9), which plays an important role in the clearance of pathogens during the immune response (18). IRF5 is a downstream regulator of the TLR-MyD88 signaling pathway (48). TLR activation results in nuclear translocation of IRF5 and induction of the proinflammatory cytokines interleukin-6 (IL-6), interleukin-12 (IL-12), and TNF-α (51). We speculate that the downregulation of IRF5 (FC = −1.7) in human monocytes responding to *N. meningitidis* could be a mechanism to reduce the detrimental proinflammatory effects induced by massive LPS stimulation. The upregulation of IRF5 (FC = 1.7) by IL-10 is seemingly inconsistent with a "pure" anti-inflammatory role of IL-10 in sepsis syndromes.

The final transcription regulator, BCL3, was upregulated by IL-10 (FC = 2.1) compared with monocytes stimulated by *N. meningitidis* alone. BCL3 has previously been shown to be induced by IL-10 and to inhibit the production of TNF-α in mouse peritoneal macrophages stimulated by *Escherichia coli* LPS (22). However, it is unclear whether BCL3 is a downstream mediator of IL-10's anti-inflammatory effect, as another study found that BCL3 could upregulate IL-10 gene expression after being upregulated in response to an autocrine factor secreted by LPS (53).

Finally, we identified genes encoding proteins with inhibitory receptor activity in the plasma membrane: LAIR1 (FC = 2.7), LILRB2 (FC = 2.2), LILRA2 (FC = 2.0), and LILRA5 (FC = 2.5) were all upregulated by IL-10 compared with monocytes stimulated by *N. meningitidis* alone. These genes belong to the leukocyte immunuglobulin-like receptor family (9) and could potentially be

TABLE 4 Network generation of genes downregulated by *N. meningitidis* and upregulated by IL-10[a]

Network	Molecules in network[b]	Score[c]	Top functions
1	**ARNTL2**, **BCL3**, BCL2L11, CCND2, CCR5, CD36, CD38, CDKN1B, CISH, **CRK**, FCER1G, **FKBP1A**, **FURIN**, **GNA15**, **HCK**, **IFNAR1**, IL-13, IL13RA1, **LDHA**, **LILRB2**, **LTBR**, MAOA, MRC1, **NLRC4**, NOS2, **PRELID1**, RAC1, RAC2, RHOA, STAT4, STAT6, **SWAP70**, SYK, TYK2, **VAV1**	16	Cell death, cellular growth and proliferation, hematological system development and function
2	**ARRDC4**, CBL, CCL2, CCL3, CCR5, CD19, CD28, CSF1R, **CTSB**, **CXCL16**, **DAB2**, **DTNBP1**, **FPR2**, FYN, GAB2, **IFNAR1**, IL-6, **IL10RB**, IL-15, **IL17RA**, **IRF5**, IRF8, KHDRBS1, LAT, PAG1, PRKCQ, PTGS2, **PTPN2**, PTPN11, **PTTG1IP**, **RASA1**, STAT3, **THBD**, TREM, **VCAN**	16	Hematological system development and function, tissue morphology and inflammatory response
3	AKT1, **ANXA4**, AP3B1, **ARRB2**, B3GNT2, CBLB, CCL1, CCL3, **CD14**, CEBPE, CHUK, CISH, **DICER1**, **FPR1**, GCLM, GSTA1, IL1A, IL1R1, IL1R2, **IL1RAP**, INPP5D, **IRAK4**, **LAIR1**, **LILRA2**, MAP3K7, NFE2L2, NFKBIB, NQ01, **PLAGL2**, SFTPD, **TLR1**, TNF, **TNFRSF1A**, TRAF2	12	Inflammatory response, cellular movement, hematological system development and function

[a] Gene networks were generated based on existing scientific information about the molecular relationships between genes and gene products in the Ingenuity Knowledge Base.
[b] Genes in bold are the focus molecules which were significantly downregulated by *N. meningitidis* and upregulated by IL-10.
[c] The score indicates the likelihood that the relationships in the network could be explained by chance alone. A score of 2 would indicate that there is a 10^{-2} probability that the focus molecules are in a network together by chance.

involved in the mediation of the IL-10 anti-inflammatory response. One study has shown that expression of LILRB2 may be increased in macrophages cultured *in vitro* with *Salmonella* Typhimurium LPS, which is contradictory with our finding (i.e., that it is downregulated with an FC of −2.0 by *N. meningitidis*) (10). LAIR1 has been shown to have an inhibitory potential on various immune cells with downregulation of cytokine production and inhibition of the differentiation of peripheral blood precursors and other steps of the immune response (35). LAIR1 has not been shown to be regulated by IL-10 according to the Ingenuity Knowledge Base and searches in the literature. LILRA2 has recently been identified to selectively inhibit LPS-mediated cytokine production (30) and to induce IL-10 secretion from monocytes (25), while LILRA5 has been shown to induce production of proinflammatory cytokines by monocytes and to be regulated by IL-10 (36).

Identification of genes upregulated in synergy by IL-10 in combination with *N. meningitidis*. We identified 53 genes (FC ≥ 1.5) that were individually upregulated by *N. meningitidis* and IL-10 and have an additional, increased expression when *N. meningitidis* stimulated the monocytes in combination with IL-10 (see Table S3 and the heatmap in Fig. S8 in the supplemental material). This synergistic effect was defined to be present when the fold changes individually induced by IL-10 and *N. meningitidis* were lower than the expression observed in combination. These genes have to our knowledge not previously been studied as a group. The molecular and cellular functions of the genes were most associated with cellular development (6 genes), lipid metabolism (3 genes), molecular transport (3 genes), small-molecule biochemistry (4 genes), and antigen presentation (5 genes). From the gene list, IPA identified a single network with the significant score of 16 (Table 5; see Fig. S9 in the supplemental material). The functions related to the molecules in the network were cell-mediated immune response, cellular development, and cellular function and maintenance. The three most upregulated genes were signaling lymphocytic activation molecule family member 1 (SLAMF1), interleukin-2 receptor alpha (IL2RA) (FC = 16.5), and Absent in Melanoma 2 (AIM2) (FC = 18.3). SLAMF1 (also known as SLAM or CD150) has been identified to be a lymphocyte-activating molecule. The expression of SLAMF1 in monocytes is induced *in vitro* by bacterium-derived ligands of TLR-2, TLR-4, and TLR-5 (14). The study of Williams et al. also showed that IL-10 and LPS induce expression of SLAMF1 (54). In our study, while SLAMF1 was induced by *N. meningitidis* (FC = 7.4) or IL-10 (FC = 9.3), the expression was vastly increased in the presence of both (FC = 54.5).

IL2RA constitutes, together with the IL2RB chain and the common gamma chain, the high-affinity receptor for IL-2. Although the gene IL2RA was markedly upregulated (FC = 16.5, versus FC = 4.3 and FC = 2.0 in *N. meningitidis* and IL-10, respectively), the role of IL-2 in meningococcal disease is probably limited, as levels in plasma are low or undetectable and differ from those of other key cytokines (5). AIM2 binds cytoplasmic DNA, engages ASC, and forms a caspase-1-activating inflammasome (50). This may lead to activation of pyroptotic cell death in cells containing caspase-1 (50), which is constitutively expressed in human monocytes (3). AIM2 is found to be involved in inflammasome activation in macrophages infected by *Listeria monocytogenes* (50). Listeria DNA, which escapes into macrophage cytoplasm, is able to trigger AIM2 oligomerization, caspase-1 activation, and pyroptosis of the cells. The AIM2 inflammasome is also implicated in

TABLE 5 Gene network induced in synergy by *N. meningitidis* in combination with IL-10[a]

Molecules in network 1[b]	Score[c]	Top functions
ADAMDEC1, **AIM2**, CASP1, CD40LG, **CMAH**, CSF2, CXCR4, CYLD, **DPP4,** ETS1, FOXP3, IFNG, **IFNK**, IL-2, IL-4, IL-5, IL-8, IL-13, IL-15, IL-21, IL-27, IL1B, **IL2RA**, **IRF2**, **MEFV**, SH2D1A, SHH, **SLAMF1**, **SOCS1**, TFGB1, **TRIP10**, ULBP1, WAS, XCL1.	16	Cell-mediated immune response, cellular development, cellular function and maintenance

[a] Gene networks were generated based on existing scientific information about the molecular relationships between genes and gene products in the Ingenuity Knowledge Base.
[b] Genes in bold are the focus molecules which were synergistically upregulated by *N. meningitidis* in combination with IL-10.
[c] The score indicates the likelihood that the relationships in the network could be explained by chance alone. A score of 2 would indicate that there is a 10^{-2} probability that the focus molecules are in a network together by chance.

TABLE 6 Comparison of microarray and qRT-PCR data for selected differentially expressed genes in human monocytes incubated with *N. meningitidis* and *N. meningitidis* combined with IL-10[a]

Gene	Affymetrix fold change		qRT-PCR relative quantity	
	N. meningitidis	*N. meningitidis* plus IL-10	*N. meningitidis*	*N. meningitidis* plus IL-10
IL-1β	1.5	1.3	14.5	5.3
TNF-α	6.2	2.0	72.5	6.7
CCL3	2.5	2.1	39.8	7.3
CXCL10	12.7	6.4	96.4	15.9
SLAMF-1	7.4	54.4	34.4	696
AIM2	9.9	18.3	18.6	71
CASP5	1.6	9.6	2.2	18.3
NLRP3	3.5	1.8	9.3	2.3
Tissue factor	7.1	1.6	44.8	4.3
RIPK2	3.7	1.4	7.0	1.6

[a] The presented fold changes and relative quantities are in comparison to monocytes incubated with vehicle. Estimations of correlation between microarray analysis and qRT-PCR showed $r = 0.7$ for *N. meningitidis* and $r = 0.95$ for *N. meningitidis* plus IL-10, using Pearson's correlation test.

infections by *Francisella tularensis* (50). In meningococcal septic shock plasma, a close correlation exists between a high concentration of meningococcal DNA, high levels of LPS, and clinical outcome (42). Intracellularly located *N. meningitidis* in neutrophils, monocytes, macrophages, and endothelial cells may possibly activate AIM2 through release of *N. meningitidis* DNA. Caspase-5, which also has been implicated with the inflammasome (33), was significantly upregulated by the combination of *N. meningitidis* and IL-10 (FC = 9.6, versus FC = 1.6 and FC = 4.4 in *N. meningitidis* and IL-10, respectively) in our experiments.

Identification of genes downregulated in synergy by *N. meningitidis* in combination with IL-10. Our gene expression profiling did not reveal genes which were significantly downregulated in synergy by *N. meningitidis* in combination with IL-10.

Validation of gene expression data from Affymetrix by qRT-PCR. To validate the gene expression data analyzed by microarray analysis, 10 differentially expressed genes were selected for quantification by qRT-PCR (Table 6). Estimations of correlation between microarray analysis and qRT-PCR showed $r = 0.7$ for *N. meningitidis* and $r = 0.95$ for *N. meningitidis* and IL-10.

Quantification of cytokines in the culture supernatant. The effects of *N. meningitidis* and IL-10 on cytokine release to the cell culture medium were also assessed. The results were compared with their corresponding mRNA levels. Of 30 cytokines measured, 6 were detectable in culture supernatants from human monocytes stimulated with *N. meningitidis*. The presence of IL-10 reduced the concentration of these cytokines by between 92% and 40% (Table 7). This shows that the presence of IL-10 has a rapid and potent inhibitory effect on the synthesis and secretion of selected proinflammatory cytokines, i.e., IL-1β, IL-6, IL-8, TNF-α, MIP-α, and MIP-β, induced by *N. meningitidis* in human monocytes.

IL-1β was reduced by 93% in the culture supernatant, while the gene expression was not significantly inhibited in the presence of IL-10. Since NLRP3 forms the inflammasome complex responsible for the activation of caspase-1 and the subsequent cleavage of pro-IL-1β (15, 26), an inhibitory effect of IL-10 on NLRP3 may be a mechanism by which IL-10 inhibits IL-1β secretion. However, determining this requires further functional studies.

The mRNA levels of IL-8 and MIP-α were not significantly inhibited by IL-10, but their concentrations in the culture supernatant were reduced by 49% and 41% in the presence of IL-10. For IL-6, TNF-α, and MIP-β, mRNA expression levels seemed to correlate well with the cytokine measurements. Several cytokines with mRNA levels upregulated by *N. meningitidis* and significantly downregulated by a combination of *N. meningitidis* and IL-10 were not detected in the culture supernatants. These were IFN-γ (FC = 16.9 versus FC = −2.6), IL-12 (FC = 13.0 versus FC = −6.5), IL-1-α (FC = 2.2 versus FC = −3.0 by IL-10), CXCL10 (FC = 12.7 versus FC = −2.0 by IL-10), IL-15 (FC = 2.5 by *N. meningitidis* and not significantly affected by IL-10), G-CSF (FC = 3.7 versus FC = −1.8 by IL-10), and GM-CSF (FC = 3.3 versus FC = −3.5 by IL-10). These findings illustrate that gene expression levels after 3 h may not adequately reflect cytokine release in human monocytes stimulated by *N. meningitidis* and IL-10 and that there are limitations of studying gene expression alone when elucidating complex cell functions.

Conclusion. The coexistence of high levels of *N. meningitidis* (10^6 cells/ml) and IL-10 (25 ng/ml), as used in our experiments, reflects a clinical situation occurring in patients with fulminant meningococcal sepsis (4, 7, 13, 27). We have shown that IL-10 has a global effect on the transcriptional profile induced by *N. meningitidis* in elutriation-purified human monocytes. IL-10 is able to

TABLE 7 Relationship between fold changes of mRNA transcripts and corresponding protein concentrations in monocyte supernatants after 3 h of incubation[a]

Cytokine	Gene expression (fold change)			Concn in culture supernatants (ng/ml)[b]				
	N. meningitidis vs control	*N. meningitidis* + IL-10 vs control	IL-10 vs control	*N. meningitidis*	*N. meningitidis* + IL-10	IL-10	Control	% reduction[c]
IL-1β	1.5	1.3	−1.3	1.8 ± 0.3	0.13 ± 0.01	N/A	0.008 ± 0.002	93%*
IL-6	6.4	2.2	NC	7.9 ± 0.6	3.20 ± 0.4	0.09 ± 0.02	0.24 ± 0.1	49%*
IL-8	1.6	1.2	−1.3	14.9 ± 1.6	6.5 ± 0.9	0.4±.05	1.4 ± 0.4	58%*
TNF-α	6.2	2.0	−2.3	10.0 ± 0.7	2.3 ± 0.2	0.04 ± 0.008	0.1 ± 0.01	36%*
MIP-α	2.5	2.1	−2.2	12.5 ± 1.6	6.6 ± 0.8	0.07 ± 0.02	0.2 ± 0.04	41%
MIP-β	3.3	2.2	−2.1	10.8 ± 1.5	5.6 ± 1.1	0.3 ± 0.1	0.9 ± 0.2	39.8%*

[a] NC, no change; N/A, below detection limit.

[b] Values are means ± standard errors of the means (five samples per group) for cytokines secreted by monocytes treated with each inducer. Means were compared with paired *t* tests.

[c] Reduction in *N. meningitidis* plus IL-10 compared with *N. meningitidis* alone. Asterisk indicates significance ($P < 0.01$ after Bonferroni correction for the number of samples) between *N. meningitidis* and *N. meningitidis* in combination with IL-10.

both inhibit and induce the expression of a large number of genes related to a variety of functions, and the transcriptional profiling revealed promising candidates for further studies of the downstream effects of IL-10. IL-10 and *N. meningitidis* were also powerful synergistic activators of gene expression in human monocytes. By using Ingenuity Pathway Analysis, we confirmed that NLRP3 and AIM2, key genes of the inflammasome, were regulated by *N. meningitidis* and IL-10. The correlation between high levels of IL-10, *N. meningitidis* LPS, and *N. meningitidis* DNA in patient shock plasma and activation of the inflammasome might thus be a new area of study to elucidate disease mechanisms in systemic meningococcal disease. Taken together, our gene expression profile and the subsequent analysis with Ingenuity Pathway Analysis suggest that the high levels of IL-10 in meningococcal septic shock plasma samples may exert a profound effect on a variety of human monocyte functions and likely on other cell types, including tissue macrophages and dendritic cells, during the rapidly evolving infection.

ACKNOWLEDGMENTS

We thank the following persons for excellent technical assistance: Anne Marie Siebke Trøseid, Blood Cell Research Unit, Section for Research, Department of Medical Biochemistry, for validation of the Affymetrix data by qRT-PCR, and Berit Nyland and Ernst Arne Höiby, National Institute of Public Health, Oslo, Norway, for providing cultures of the reference strain *N. meningitidis* 44/76.

REFERENCES

1. **Antoniv TT, Park-Min K-H, Ivashkiv LB.** 2005. Kinetics of IL-10-induced gene expression in human macrophages. Immunobiology **210**: 87–95.
2. **Asadullah K, Sterry W, Volk HD.** 2003. Interleukin-10 therapy—review of a new approach. Pharmacol. Rev. **55**:241–269.
3. **Ayala JM, et al.** 1994. IL-1 beta-converting enzyme is present in monocytic cells as an inactive 45-kDa precursor. J. Immunol. **153**:2592–2599.
4. **Bjerre A, Brusletto B, Øvstebø R, Joø GB, Kierulf P, Brandtzaeg P.** 2003. Identification of meningococcal LPS as a major monocyte activator in IL-10 depleted shock plasmas and CSF by blocking the CD14-TLR4 receptor complex. J. Endotoxin Res. **9**:155–163.
5. **Brandtzaeg P, Bjerre A, Øvstebø R, Brusletto B, Joø GB, Kierulf P.** 2001. Neisseria meningitidis lipopolysaccharides in human pathology. J. Endotoxin Res. **7**:401–420.
6. **Brandtzaeg P, et al.** 1989. Plasma endotoxin as a predictor of multiple organ failure and death in systemic meningococcal disease. J. Infect. Dis. **159**:195–204.
7. **Brandtzaeg P, et al.** 1996. Net inflammatory capacity of human septic shock plasma evaluated by a monocyte-based target cell assay: identification of interleukin-10 as a major functional deactivator of human monocytes. J. Exp. Med. **184**:51–60.
8. **Brandtzaeg P, Ovstebø R, Kierulf P.** 1992. Compartmentalization of lipopolysaccharide production correlates with clinical presentation in meningococcal disease. J. Infect. Dis. **166**:650–652.
9. **Brown D, Trowsdale J, Allen R.** 2004. The LILR family: modulators of innate and adaptive immune pathways in health and disease. Tissue Antigens **64**:215–225.
10. **Brown DP, et al.** 2009. The inhibitory receptor LILRB4 (ILT3) modulates antigen presenting cell phenotype and, along with LILRB2 (ILT4), is upregulated in response to Salmonella infection. BMC Immunol. **10**:56.
11. **Chin AI, et al.** 2002. Involvement of receptor-interacting protein 2 in innate and adaptive immune responses. Nature **416**:190–194.
12. **Couper KN, Blount DG, Riley EM.** 2008. IL-10: the master regulator of immunity to infection. J. Immunol. **180**:5771–5777.
13. **Derkx B, Marchant A, Goldman M, Bijlmer R, van Deventer S.** 1995. High levels of interleukin-10 during the initial phase of fulminant meningococcal septic shock. J. Infect. Dis. **171**:229–232.
14. **Farina C, Theil D, Semlinger B, Hohlfeld R, Meinl E.** 2004. Distinct responses of monocytes to Toll-like receptor ligands and inflammatory cytokines. Int. Immunol. **16**:799–809.
15. **Gross O, Thomas CJ, Guarda G, Tschopp J.** 2011. The inflammasome: an integrated view. Immunol. Rev. **243**:136–151.
16. **He C-X, Prevot N, Boitard C, Avner P, Rogner UC.** 2010. Inhibition of type 1 diabetes by upregulation of the circadian rhythm-related aryl hydrocarbon receptor nuclear translocator-like 2. Immunogenetics **62**:585–592.
17. **Hochart H, et al.** 2008. Concentration-dependent roles for heparin in modifying lipopolysaccharide-induced activation of mononuclear cells in whole blood. Thromb. Haemost. **99**:570–575.
18. **Honda K, Taniguchi T.** 2006. IRFs: master regulators of signalling by Toll-like receptors and cytosolic pattern-recognition receptors. Nat. Rev. Immunol. **6**:644–658.
19. **Høgåsen AK, Abrahamsen TG.** 1995. Heparin suppresses lipopolysaccharide-induced monocyte production of several cytokines, but simultaneously stimulates C3 production. Thromb. Res. **80**:179–184.
20. **Janský L, Reymanová P, Kopecký J.** 2003. Dynamics of cytokine production in human peripheral blood mononuclear cells stimulated by LPS or infected by Borrelia. Physiol. Res. **52**:593–598.
21. **Katzav S.** 2009. Vav1: a hematopoietic signal transduction molecule involved in human malignancies. Int. J. Biochem. Cell Biol. **41**:1245–1248.
22. **Kuwata H, et al.** 2003. IL-10-inducible Bcl-3 negatively regulates LPS-induced TNF-alpha production in macrophages. Blood **102**:4123–4129.
23. **Landrette SF, et al.** 2005. Plag1 and Plagl2 are oncogenes that induce acute myeloid leukemia in cooperation with Cbfb-MYH11. Blood **105**: 2900–2907.
24. **Lang R, Patel D, Morris JJ, Rutschman RL, Murray PJ.** 2002. Shaping gene expression in activated and resting primary macrophages by IL-10. J. Immunol. **169**:2253–2263.
25. **Lee DJ, et al.** 2007. LILRA2 activation inhibits dendritic cell differentiation and antigen presentation to T cells. J. Immunol. **179**:8128–8136.
26. **Leemans JC, Cassel SL, Sutterwala FS.** 2011. Sensing damage by the NLRP3 inflammasome. Immunol. Rev. **243**:152–162.
27. **Lehmann AK, Halstensen A, Sørnes S, Røkke O, Waage A.** 1995. High levels of interleukin 10 in serum are associated with fatality in meningococcal disease. Infect. Immun. **63**:2109–2112.
28. **Livak KJ, Schmittgen TD.** 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods **25**: 402–408.
29. **Lu C, et al.** 2005. Participation of Rip2 in lipopolysaccharide signaling is independent of its kinase activity. J. Biol. Chem. **280**:16278–16283.
30. **Lu HK, et al.** 2012. LILRA2 selectively modulates LPS-mediated cytokine production and inhibits phagocytosis by monocytes. PLoS One **7**:e33478. doi:10.1371/journal.pone.0033478.
31. **Lund PK, Joø GB, Westvik AB, Ovstebø R, Kierulf P.** 2000. Isolation of monocytes from whole blood by density gradient centrifugation and counter-current elutriation followed by cryopreservation: six years' experience. Scand. J. Clin. Lab. Invest. **60**:357–365.
32. **Mariathasan S, et al.** 2004. Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf. Nature **430**:213–218.
33. **Martinon F, Burns K, Tschopp J.** 2002. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol. Cell **10**:417–426.
34. **Mazzoccoli G, et al.** 2012. ARNTL2 and SERPINE1: potential biomarkers for tumor aggressiveness in colorectal cancer. J. Cancer Res. Clin. Oncol. **138**:501–511.
35. **Meyaard L.** 2008. The inhibitory collagen receptor LAIR-1 (CD305). J. Leukoc. Biol. **83**:799–803.
36. **Mitchell A, et al.** 2008. LILRA5 is expressed by synovial tissue macrophages in rheumatoid arthritis, selectively induces pro-inflammatory cytokines and IL-10 and is regulated by TNF-alpha, IL-10 and IFN-gamma. Eur. J. Immunol. **38**:3459–3473.
37. **Murray PJ.** 2005. The primary mechanism of the IL-10-regulated antiinflammatory response is to selectively inhibit transcription. Proc. Natl. Acad. Sci. U. S. A. **102**:8686–8691.
38. **Murray PJ.** 2006. Understanding and exploiting the endogenous interleukin-10/STAT3-mediated anti-inflammatory response. Curr. Opin. Pharmacol. **6**:379–386.
39. **Nembrini C, et al.** 2009. The kinase activity of Rip2 determines its stability and consequently Nod1- and Nod2-mediated immune responses. J. Biol. Chem. **284**:19183–19188.
40. **Netea MG, et al.** 2009. Differential requirement for the activation of the inflammasome for processing and release of IL-1beta in monocytes and macrophages. Blood **113**:2324–2335.

41. **O'Garra A, Barrat FJ, Castro AG, Vicari A, Hawrylowicz C.** 2008. Strategies for use of IL-10 or its antagonists in human disease. Immunol. Rev. **223**:114–131.
42. **Ovstebø R, et al.** 2004. Use of robotized DNA isolation and real-time PCR to quantify and identify close correlation between levels of Neisseria meningitidis DNA and lipopolysaccharides in plasma and cerebrospinal fluid from patients with systemic meningococcal disease. J. Clin. Microbiol. **42**:2980–2987.
43. **Ovstebø R, et al.** 2008. Identification of genes particularly sensitive to lipopolysaccharide (LPS) in human monocytes induced by wild-type versus LPS-deficient Neisseria meningitidis strains. Infect. Immun. **76**:2685–2695.
44. **Park J-H, et al.** 2007. RICK/RIP2 mediates innate immune responses induced through Nod1 and Nod2 but not TLRs. J. Immunol. **178**:2380–2386.
45. **Piehler AP, Grimholt RM, Ovstebø R, Berg JP.** 2010. Gene expression results in lipopolysaccharide-stimulated monocytes depend significantly on the choice of reference genes. BMC Immunol. **11**:21.
46. **Rietschel ET, et al.** 1996. Bacterial endotoxin: chemical constitution, biological recognition, host response, and immunological detoxification. Curr. Top. Microbiol. Immunol. **216**:39–81.
47. **Rojahn A, Brusletto B, Øvstebø R, Haug KBF, Kierulf P, Brandtzaeg P.** 2008. Does granulocyte colony-stimulating factor ameliorate the proinflammatory response in human meningococcal septic shock? Crit. Care Med. **36**:2583–2589.
48. **Sabat R, et al.** 2010. Biology of interleukin-10. Cytokine Growth Factor Rev. **21**:331–344.
49. **Stephens DS, Greenwood B, Brandtzaeg P.** 2007. Epidemic meningitis, meningococcaemia, and Neisseria meningitidis. Lancet **369**:2196–2210.
50. **Strowig T, Henao-Mejia J, Elinav E, Flavell R.** 2012. Inflammasomes in health and disease. Nature **481**:278–286.
51. **Takaoka A, et al.** 2005. Integral role of IRF-5 in the gene induction programme activated by Toll-like receptors. Nature **434**:243–249.
52. **van Deuren M, Brandtzaeg P, van der Meer JW.** 2000. Update on meningococcal disease with emphasis on pathogenesis and clinical management. Clin. Microbiol. Rev. **13**:144–166.
53. **Wessells J, et al.** 2004. BCL-3 and NF-kappaB p50 attenuate lipopolysaccharide-induced inflammatory responses in macrophages. J. Biol. Chem. **279**:49995–50003.
54. **Williams L, Jarai G, Smith A, Finan P.** 2002. IL-10 expression profiling in human monocytes. J. Leukoc. Biol. **72**:800–809.