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An mRNA atlas of G protein-coupled receptor expression during primary human monocyte/macrophage differentiation and lipopolysaccharide-mediated activation identifies targetable candidate regulators of inflammation

Daniel M. Hohenhaus[a,b], Kolja Schaale[a,b], Kim-Anh Le Cao[a,c], Vernon Seow[a], Abishek Iyer[a], David P. Fairlie[a,b], Matthew J. Sweet[a,b,*]

[a] Institute for Molecular Bioscience, The University of Queensland, Brisbane, Qld 4072, Australia
[b] Australian Infectious Disease Research Centre, The University of Queensland, Brisbane, Qld 4072, Australia
[c] Queensland Facility for Advanced Bioinformatics, The University of Queensland, Brisbane, Qld 4072, Australia

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ABSTRACT

G protein-coupled receptors (GPCRs) are among the most important targets in drug discovery. In this study, we used TaqMan Low Density Arrays to profile the full GPCR repertoire of primary human macrophages differentiated from monocytes using either colony stimulating factor-1 (CSF-1/M-CSF) (CSF-1 Mϕ) or granulocyte macrophage colony stimulating factor (GM-CSF) (GM-CSF Mϕ). The overall trend was a downregulation of GPCRs during monocyte to macrophage differentiation, but a core set of 10 genes (e.g. *LGR4*, *MRGPRF* and *GPR143*) encoding seven transmembrane proteins were upregulated, irrespective of the differentiating agent used. Several of these upregulated GPCRs have not previously been studied in the context of macrophage biology and/or inflammation. As expected, CSF-1 Mϕ and GM-CSF Mϕ exhibited differential inflammatory cytokine profiles in response to the Toll-like Receptor (TLR)4 agonist lipopolysaccharide (LPS). Moreover, 15 GPCRs were differentially expressed between these cell populations in the basal state. For example, *EDG1* was expressed at elevated levels in CSF-1 Mϕ versus GM-CSF Mϕ, whereas the reverse was true for *EDG6*. 101 GPCRs showed differential regulation over an LPS time course, with 65 of these profiles being impacted by the basal differentiation state (e.g. *GPRC5A*, *GPRC5B*). Only 14 LPS-regulated GPCRs showed asynchronous behavior (divergent LPS regulation) with respect to differentiation status. Thus, the differentiation state primarily affects the magnitude of LPS-regulated expression, rather than causing major reprogramming of GPCR gene expression profiles. Several GPCRs showing differential profiles between CSF-1 Mϕ and GM-CSF Mϕ (e.g. *P2RY8*, *GPR92*, *EMR3*) have not been widely investigated in macrophage biology and inflammation. Strikingly, several closely related GPCRs displayed completely opposing patterns of regulation during differentiation and/or activation (e.g. *EDG1* versus *EDG6*, *LGR4* versus *LGR7*, *GPRC5A* versus *GPRC5B*). We propose that selective regulation of *GPCR5A* and *GPCR5B* in CSF-1 Mϕ contributes to skewing toward the M2 macrophage phenotype. Our analysis of the GPCR repertoire expressed during primary human monocyte to macrophage differentiation and TLR4-mediated activation provides a valuable new platform for conducting future functional analyses of individual GPCRs in human macrophage inflammatory pathways.

Introduction

The human genome encodes more than 800 seven transmembrane-containing proteins, of which more than 300 are non-olfactory receptors (Alexander et al., 2011). The majority of these receptors transduce signals, at least in part, by coupling to heterotrimeric G proteins. Thus, the term G protein-coupled receptor (GPCR) is often used interchangeably with seven transmembrane-containing receptor, although this is not always the case. For simplicity, we hereafter refer to all seven

Abbreviations: CSF-1, colony stimulating factor-1; CSF-1 Mϕ, monocytes differentiated to HMDM with CSF-1; GM-CSF, granulocyte macrophage colony stimulating factor; GM-CSF Mϕ, monocytes differentiated to HMDM with GM-CSF; GPCR, G protein-coupled receptor; HMDM, human monocyte-derived macrophage; HSC, hematopoietic stem cell; IL, interleukin; LPS, lipopolysaccharide; TLDA, TaqMan low density arrays; TLR, Toll-like receptor; TNF, tumor necrosis factor.

* Corresponding author at: Institute for Molecular Bioscience, University of Queensland, St Lucia 4072, Australia. Tel.: +61 7 3346 2082; fax: +61 7 3346 2101.
E-mail address: m.sweet@imb.uq.edu.au (M.J. Sweet).

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transmembrane-containing proteins as GPCRs. GPCRs have been sub-divided according to the GRAFS classification system into the glutamate, rhodopsin, adhesion, frizzled/taste2 and secretin GPCR sub-families (Fredriksson et al., 2003). The International Union of Basic and Clinical Pharmacology Committee on Receptor Nomenclature and Drug Classification alternatively classifies GPCRs into class A (rhodopsin), class B (secretin), class C (glutamate), frizzled, and other 7TM proteins. GPCRs are the most prevalent signal-transducing proteins on the cell surface, and respond to a diverse array of environmental cues to control numerous physiological and pathological processes. They represent targets for approximately one third of all pharmaceuticals (Overington et al., 2006). With the recent emergence of solved three dimensional structures for several class A GPCRs (Venkatakrishnan et al., 2013), this family of cell surface proteins promises many new opportunities for development of therapeutic agents.

Macrophages are key innate immune cells that occupy distinct anatomical locations within every tissue of the body, and are recruited in further numbers to specific tissues when homeostasis is dysregulated (Hume, 2008). As danger sentinels, macrophages initiate inflammatory responses to enable a coordinated physiological response to limit tissue damage and initiate and coordinate repair processes. Macrophages recognize danger through several families of pattern recognition receptors such as the Toll-like receptors (TLRs) that detect both pathogen-associated molecular patterns during infections, as well as host-derived damage-associated molecular patterns that are indicative of cell stress and/or damage during acute and chronic diseases. For example, TLR4 recognizes lipopolysaccharide (LPS) from Gram-negative bacterial cell walls (Rossol et al., 2011), as well as several host-derived factors including HMGB1 (Yang et al., 2010), oxidized LDL and beta-amyloid peptide (Stewart et al., 2010). TLRs primarily act, though not exclusively, by regulating the expression of a suite of genes that control inflammation, antimicrobial responses and antigen presentation.

Recent evidence suggests that tissue resident macrophage populations can arise through both hematopoietic stem cell (HSC)-dependent and -independent processes. The latter pathway occurs independently of the c-Myb transcription factor and involves the development of some tissue macrophage populations from embryonic macrophages in the yolk sac (Schulz et al., 2012). The former pathway involves the cytokine-mediated differentiation of HSC into circulating monocytes, and of these cells into tissue resident and inflammatory macrophages. Several cytokines, including IL-3, GM-CSF, CSF-1 and IL-34, direct monocyte/macrophage differentiation from HSC. CSF-1 and IL-34 are expressed widely, but differentially, in adult mouse tissues (Wei et al., 2010), and are required for the differentiation of many tissue resident macrophage populations (Wang et al., 2012; Yoshida et al., 1990). During acute and chronic inflammation, several other cytokines including tumor necrosis factor (TNF) (Sade-Feldman et al., 2013), interferon-γ (Schroder et al., 2004) and GM-CSF (Hamilton, 2008) can also influence myeloid development. In terms of macrophage differentiation, GM-CSF is probably the most widely studied of these factors. Comparisons between GM-CSF- and CSF-1-differentiated macrophages are often made in the context of classically activated "M1" macrophages and alternatively activated "M2" macrophages, respectively (Hamilton, 2008; Hamilton and Achuthan, 2013). M1 macrophages are associated with pro-inflammatory and microbicidal responses, and are usually generated *in vitro* using IFN-γ plus LPS. In contrast, M2 macrophages are associated with anti-inflammatory responses, tissue remodeling and immunoregulation and are typically generated *in vitro* using IL-4 or IL-13 (Biswas et al., 2012). The extensive phenotypic diversity and plasticity of macrophages means that this classification system is probably overly simplistic, but it is certainly the case that GM-CSF is typically present at elevated levels during inflammatory responses, and that GM-CSF-differentiated macrophages have a hyper-inflammatory phenotype. For example, in *in vitro* experiments, mouse bone marrow-derived macrophages differentiated with GM-CSF display elevated LPS-triggered inflammatory responses, as compared to those differentiated with CSF-1 (Fleetwood et al., 2007). Increased pro-inflammatory responses have also been reported with primary human monocytes differentiated into macrophages with GM-CSF versus CSF-1 (Lacey et al., 2012; Sierra-Filardi et al., 2010; Verreck et al., 2004). Furthermore, many studies have demonstrated pathological roles for this cytokine in inflammatory diseases (Hamilton, 2008; Hamilton and Anderson, 2004), and in acute inflammatory models such as LPS-induced lung inflammation (Bozinovski et al., 2002). Thus, GM-CSF Mϕ can be considered as representative of inflammatory macrophages. In contrast, CSF-1 Mϕ are probably reasonable cellular surrogates of tissue resident macrophages under homeostatic conditions, since CSF-1 is constitutively present *in vivo* during homeostasis.

We previously documented the constitutive and LPS-regulated GPCR repertoire of mouse macrophages (Lattin et al., 2008). This analysis identified several members of the P2RY family of GPCRs that were enriched in macrophages and/or regulated by LPS. More recently however, we identified widespread divergence in LPS-regulated gene expression between primary human and mouse macrophages (Schroder et al., 2012). Others have also reported human versus mouse differences in the basal gene expression programs of monocyte subsets (Ingersoll et al., 2010), and in CSF-1 and GM-CSF differentiated macrophages (Lacey et al., 2012). Such differences may contribute to the very different transcriptomic responses in mouse models versus human inflammatory diseases (Seok et al., 2013). In light of such species differences and the need to understand GPCR expression profiles and functions in normal and pathological immune responses, we conducted a detailed expression analysis of the non-sensory GPCR repertoire expressed during primary human monocyte to macrophage differentiation and their activation by the TLR4 agonist, LPS. Our analysis has identified a small set of GPCRs that are upregulated during monocyte to macrophage differentiation, as well as numerous GPCRs showing differential expression patterns between CSF-1 Mϕ and GM-CSF Mϕ. Our data provide insights into the differential inflammatory profiles of these macrophage populations, and identifies several GPCRs not previously associated with inflammation and/or macrophage biology (e.g. LGR4, MRGPRF). This information is expected to be a valuable resource for future functional analysis of human macrophage-expressed GPCRs.

Materials and methods

Cell culture and reagents

All studies using primary human cells were approved by the University of Queensland Medical Research Ethics Committee. Human $CD14^+$ monocytes were isolated from buffy coat provided by the Australian Red Cross Blood Service using MACS $CD14^+$ positive selection kits (Miltenyi Biotech), according to the manufacturer's instructions. $CD14^+$ monocytes were cultured overnight on 10 cm TC plates (Nunc) prior to RNA extraction. Human monocyte-derived macrophages (HMDM) were generated by 7-day *in vitro* culture of $CD14^+$ monocytes in the presence of either 1×10^4 U/mL recombinant human CSF-1 (Chiron) (CSF-1 Mϕ) or 10 ng/mL recombinant human GM-CSF (Peprotech) (GM-CSF Mϕ). Both $CD14^+$ monocytes and HMDMs were maintained in Iscove's modified Dulbecco's medium (Life Technologies), supplemented with 10% heat-inactivated fetal calf serum (Life Technologies), 20 U/mL penicillin (Life Technologies), 20 μg/mL streptomycin (Life