Molecular and Cellular Endocrinology 406 (2015) 49–59

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Retinoic acid potentiates inflammatory cytokines in human mast cells: Identification of mast cells as prominent constituents of the skin retinoid network

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ARTICLE INFO

Article history:
Received 9 June 2014
Received in revised form 17 January 2015
Accepted 18 February 2015
Available online 25 February 2015

Keywords:
Mast cells
Retinoids
Retinoic acid
Retinoic acid receptor
Skin
Cytokines

ABSTRACT

Retinoic acid (RA), the active vitamin-A-metabolite, has well-established functions in skin homeostasis and in the immune system. Skin mast cells (MCs) combine traits of both structures, being of hematopoietic origin, but functional in the skin environment. It remains largely unknown whether mature MCs are targeted by the retinoid network. Here, we demonstrate that human skin MCs display substantial susceptibility to RA by which they are instructed to increase pro-inflammatory mediators (IL-1β, IL-8, TNF-α) but not histamine release. The effects are observed at physiological RA levels, in different microenvironments, and are largely donor-independent. RA susceptibility is owed to the cells' abundant expression of RARA, the receptor mediating MC cytokine responses. Unexpectedly, bioinformatics calculations on the FANTOM5 expression atlas revealed general enrichment of retinoid network components in MCs against other skin cells, and MCs rapidly upregulated RA responsive genes. In conclusion, MCs are important yet hitherto overlooked retinoid targets in the skin.

1. Introduction

Vitamin A and its active derivatives, in particular the hormonal form (all-trans) retinoic acid (abbreviated RA), regulate gene networks involved in a plethora of processes ranging from organogenesis and tissue homeostasis during vertebrate development to the regulation of cellular growth, apoptosis, differentiation, metabolism and function (Germain et al., 2006). Retinoids are also implicated in host resistance to infections, and vitamin A deficiency has been linked to increased mortality and morbidity from diverse infectious diseases like measles and diarrheal infections (Semba and Vitamin, 1999). Over the last few years, the mechanistic details underlying the immune-related functions of RA have begun to be unraveled. In particular, RA was shown to act at critical checkpoints in the adaptive immune system, by regulating the differentiation and homing ability of CD4+ cells, especially of the regulatory T cell subset (Cassani et al., 2012; Hall et al., 2011; Mucida et al., 2007), and by stimulating B cell differentiation and isotype switching to IgA (Mora, 2008). In addition, RA was uncovered to modulate the function of myeloid immune cells including dendritic cells and macrophages, thereby aiding in combating diseases like tuberculosis (Geissmann et al., 2003; Manicassamy and Pulendran, 2009; Mohty et al., 2003; Tao et al., 2006; Wheelwright et al., 2014).

An important resident immune cell subset of peripheral tissues is the mast cell (MC), which belongs to the myeloid arm of the hematopoietic system, but completes maturation only in direct contact with the target tissue after progenitor recruitment through the circulation. MCs are believed to assist in the defense against intruding pathogens and to perform immunoregulatory functions, integrating the information from the invading microbes and the tissue microenvironments to mount appropriate immune responses (Galli and Tsai, 2010; Hofmann and Abraham, 2010).

The skin, an organ particularly rich in MCs even in the absence of infection or inflammation, has long been known as an important target of RA and is well-equipped with proteins involved in RA metabolism and function (Fisher and Voorhees, 1996; Mihaly et al., 2011). Therefore, MCs in the skin are constantly exposed to RA, although the consequences of this interaction have remained largely obscure. While RA has been reported by us and others to exert modulatory effects on immature/proliferating MC subsets (mainly on

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http://dx.doi.org/10.1016/j.mce.2015.02.019
0303-7207/

adhesion molecules (Babina et al., 1997a, 1997b, 2001) and cell cycle regulators (Kinoshita et al., 2000)), the question of whether key functional programs of mature, non-proliferating MCs are targeted by RA has remained unanswered to date. This is important in view that proliferating normal MCs and MC lines differ profoundly from their quiescent counterparts in the tissue (Guhl et al., 2010; Motakis et al., 2014). One study reported that skin MC density remains unchanged or is slightly increased following application of an RA-containing cream to human skin (Hjertson et al., 2003). However, these immunohistochemical studies could not reveal possible functional changes in the MCs. An important way by which MCs fulfill biological actions is by the release of soluble mediators such as cytokines (Bischoff, 2007). Previous studies from our laboratory demonstrated that human skin MCs produce a cytokine profile, which is mainly composed of pro-inflammatory mediators, somewhat resembling that of monocytes/macrophages (Babina et al., 2004; Guhl et al., 2005, 2012). Here, we asked whether RA could impact MC functional programs. We found that MCs are instructed by RA to increase their pro-inflammatory potential, and that this phenomenon is found in different microenvironments and in MCs from a majority of human subjects. The positive impact on cytokine expression was not accompanied by altered degranulation, a substantially more rapid process. We also found a reasonable explanation for the exquisite sensitivity of skin MCs to RA, which appear to stem from the particularly high expression of the RARA gene in these cells. This could be detected by contrasting the MC transcriptome against the novel mRNA expression atlas from the FANTOM5 consortium, the most comprehensive transcriptome collection of the human body to date (Forrest et al., 2014). Intrigued by this unexpected finding we analyzed the expression of genes related to the entire network with the result that its components were enriched in MCs vis-à-vis other skin constituents. Together, our study implies that RA possesses a physiologically relevant mode of operation by affecting functional programs of fully differentiated MCs and, conversely, identifies mature MCs as significant, but hitherto barely considered targets of RA in the skin.

2. Materials and methods

2.1. Cells

MC purification from human foreskin was performed as described previously (Babina et al., 2004; Guhl et al., 2010). The skin was obtained from circumcisions with informed consent of the patients or their legal guardians. All experiments were conducted according to the Declaration of Helsinki Principles and approved by the Ethics Committee of the Charité Universitätsmedizin Berlin. Briefly, skin was cut into strips and treated with dispase (Becton Dickinson, Heidelberg, Germany) at 4 °C overnight. After removal of the epidermis, the dermis was chopped into small pieces and digested with collagenase (Worthington, Lakewood, NJ) for 1 h at 37 °C. After three steps of filtration, MC purification from the dispersates was achieved by selection with microbeads coupled to an anti-human c-Kit antibody (Miltenyi Biotec, Bergisch Gladbach, Germany) and an Auto-MACS separation device, as described (Babina et al., 2004; Guhl et al., 2010). Viability (trypan blue exclusion) and MC purity (acidic toluidine-blue staining) always exceeded 99%, as reported and demonstrated previously (Guhl et al., 2010; Motakis et al., 2014).

Fibroblasts and keratinocytes (used as controls) were purified from the dermis and epidermis, respectively, as described (Artuc et al., 2002). The human mast cell line HMC-1 (kindly provided by Dr. J.H. Butterfield) (Butterfield et al., 1988), shown previously by us to be responsive to RA (Babina et al., 1997a, 1997b, 2001), was included as an additional control in several experiments.

2.2. Mast cell treatment

Skin MCs were used directly *ex vivo* and kept in basal Iscove's medium with standard medium (10% FCS and antibiotics unless specified otherwise) at 1×10^6/ml for 24 h in the presence or absence of *all-trans* RA (Sigma-Aldrich, Steinheim, Germany) at the concentrations specified in the text and figures. In one series of experiments, MCs obtained from different subjects were treated with RA individually, in a second series MCs were pooled from several donors, and then pretreated (or not) with RA for 1 h prior to stimulation by either PMA (Phorbol 12-myristate-13-acetate, Sigma-Aldrich) at 25 ng/ml or anti-IgE (1:20,000, mouse IgM-antihuman IgE Fc fragment Calbiochem-Novabiochem, Bad Soden, Germany). Control cells remained without stimulation. The importance of caspase-1 activity for MC IL-1β production was studied by applying the inhibitor Z-YVAD-FMK (Biovision, BioCat, Heidelberg, Germany) at 2 μM. Cells were harvested after 24 h for collection of supernatants and lysates, exactly as described (Babina et al., 2004) and immediately frozen at –80 °C for cytokine determination. For mechanistic experiments, the RAR specific agonists CD336, CD367, and CD2019 were used at 1 μM (kind gift of Dr. U. Reichert, CIRD Galderma, Sophia Antipolis, France) and compared directly to RA.

2.3. ELISA

Cytokines were quantified by using specific ELISA kits (R&D Systems, Wiesbaden, Germany), according to the vendor's specifications, exactly as described (Babina et al., 2004).

2.4. Histamine release assay

Histamine release was assessed, as described previously (Babina et al., 2004; Guhl et al., 2010, 2014). In brief, MCs were re-suspended in PAG–CM [PAG–CM = piperazine-*N*,*N*-bis[2-ethanesulfonic acid]–albumin–glucose buffer containing 3 mM $CaCl_2$ and 1.5 mM $MgCl_2$, pH 7.4] at 1×10^5/ml, pretreated (or not) with RA (at 100 nM, 30 min) and challenged with mouse IgM anti-human IgE (Calbiochem, Bad Soden, Germany) at 1:20,000 for additional 30 min at 37 °C or kept in PAG–CM for spontaneous release. Supernatants were stored at –20 °C until measurement. For total histamine content, MCs were lysed in 1% perchloric acid for 30 min at 37 °C centrifuged and the cell free supernatants were stored at –20 °C. Quantification of histamine content was performed by an automated fluorescence method, using an autoanalyzer (Borgwald Technik, Hamburg, Germany) referring to a 5-point histamine standard curve. All histamine determinations were performed in triplicate.

2.5. Conventional and quantitative RT-PCR

RT-PCR was performed as described (Babina et al., 2001, 2005; Guhl et al., 2010). Briefly, total RNA was isolated using the Nucleo spin RNA Kit (Macherey-Nagel, Düren, Germany). Expression of retinoid receptors (RARA, RARB, RARG, RXRA, RXRB, RXRG) in skin MCs was analyzed by conventional RT-PCR, using the primer pairs and conditions described previously (Babina et al., 2001), relative to control cells. Quantitative PCR was carried out with the LC Fast Start DNA Master SYBR Green kit (Roche Applied Science) (Babina et al., 2005; Guhl et al., 2010). The primer pairs were as follows: for RARA: 5′-CTATGCTGGGTGGACTCTCC and 5′-GAACTGCTGCTCTGGGTCTC; for RARB 5′-CTGCCTTTGGAAATGGATGA and 5′-TGCTGGGTCGTCTTTTTCTG; for RXRB 5′-CCCTACTCTCAGCCAGGGAT and 5′-TGCATTTCTTTTCGCACCCC; for CD43: 5′-TGCTGGTGGTAAGCCCAGA and 5′-CAGTGCTGGCACCAATGGA, and for β actin 5′-CTGGAACGGTGAAGGTGACA and 5′-AAGGGACTTCCTGTAACAATGCA. Values were normalized to the housekeeping gene β actin.