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Expression of complement components, receptors and regulators by human dendritic cells

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ABSTRACT

Integration of innate and adaptive arms of the immune response at a cellular and molecular level appears to be fundamental to the development of powerful effector functions in host defence and aberrant immune responses. Here we provide evidence that the functions of human complement activation and antigen presentation converge on dendritic cells (DCs). We show that several subsets of human DCs [i.e., monocyte derived ($CD1a^+CD14^-$), dermal ($CD1a^+$DC-$SIGN^+$), Langerhans ($CD1a^+$Langerin$^+$), myeloid ($CD1c^+CD19^-$), plamacytoid ($CD45RA^+CD123^+$)] express many of the components of the classical and alternative and terminal pathways of complement. Moreover human DCs have receptors known to detect the biologically active peptides C3a and C5a (C3aR, C5aR) and the covalently bound fragments C3b and metabolites iC3b and C3d which serve in immune adhesion (i.e., CR3, CR4, CRIg). We also show that the human DC surface is characterised by membrane bound regulators of complement activation, which are also known to participate in intracellular signalling (i.e., CD46, CD55, CD59). This work provides an extensive description of complement components relevant to the integrated actions of complement and DC, illuminated by animal studies. It acts as a resource that allows further understanding and exploitation of role of complement in human health and immune mediated diseases.

1. Introduction

Dendritic cells (DCs) and the complement system are two of the most important components of innate immunity, one being a cellular component and the other a humoral constituent. Recent research has shown that complement can influence the adaptive immune response by modulating DC function and thus regulating antigen specific T cell responses. In addition, local production and activation of complement has been suggested to be critical for DC functional development (Peng et al., 2006, 2008; Li et al., 2008; Baruah et al., 2009; Strainic et al., 2008).

DCs are potent antigen presenting cells (APCs) with the unique capacity to initiate the primary immune response. In addition to their role in local innate immune responses, DCs play a critical role in the adaptive immune response by priming the immune response or by inducing tolerance (Steinman, 2006). In humans, DCs with different phenotypes are distributed throughout the body and reside at the site of potential pathogen entry or tissue injury, where they differentiate into immature DCs. The diverse functions of DCs are dependent not only on their subsets but also on their state of activation which can be regulated by many mediators including exogenous and endogenous factors (Banchereau et al., 2000; Steinman, 2003).

The complement system consists of approximately 30 distinct proteins, including pathway components, receptors and regulators. The pathway components are a group of plasma proteins [e.g. C1–C9, factor B, factor D, mannose binding lectin (MBL) and MBL-associated serine proteases (MASPs)] which participate in complement activation through which complement effector molecules (e.g. C3b, C3a/C5a, C5b-9) are generated. The receptors are membrane proteins (e.g. CR1, CR2, CR3, CR4, C3aR and C5aR) which interact with their respective effector molecules. The regulators [e.g. factor I (fI), factor H (fH), properdin (fP), CD46, CD55, CD59] are either present in soluble form or expressed on cells, and are able to mediate several regulatory mechanisms to prevent tissue damage caused by complement activation. Complement receptors and membrane regulators have a wide cellular distribution (e.g. parenchymal cells and leukocytes including lymphocytes), whereas most of the soluble circulating complement components and reg-

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ulators are thought to be mainly synthesised in the liver (Walport, 2001). However, extrahepatic production of complement has been shown in a variety of tissues/organs/cells which synthesise a number of complement proteins, either constitutively or in response to noxious stimuli (Li et al., 2007).

Although extrahepatic synthesis of complement has been widely studied in most cells and tissues, information on DCs has only recently emerged and most work has been confined to murine DCs (van Kooten et al., 2008). Previous studies have shown that murine bone-marrow derived DCs (BMDCs) are able to synthesise a range of complement pathway components, regulators and receptors and this synthesis has a substantial impact on DC activation, affecting DC functions in antigen presentation and allospecific T cell stimulation (Peng et al., 2006, 2008; Strainic et al., 2008). APCs from C1q-, C3-, fB- and fD-deficient mice exhibited a less activated phenotype and had a reduced capacity to stimulate antigen specific T cells (Peng et al., 2006; Li et al., 2008; Zhou et al., 2006; Heeger et al., 2005; Ghannam et al., 2008). In addition, DCs from C3aR or C5aR deficient mice have a similar activation and functional phenotype to the above complement component deficient mice, suggesting that C3aR- or C5aR-ligand interactions play a critical role in modulating DC activation and function (Li et al., 2008; Peng et al., 2008, 2009). These studies highlight the importance of local production of complement by APCs in modulating DC activation and function.

Although cell-autonomous expression of complement pathway components and receptors and regulators and their functional consequences have been documented in murine BMDCs, such information in human DCs is limited. Previous studies in man have found that several pathway components and regulators (e.g. C1q, C3, fI, fB, CR1) are expressed in monocyte-derived DCs (moDCs) (Castellano et al., 2004; Reis et al., 2006, 2007, 2008); and C1q and C3 can influence DC activation and maturation (Nauta et al., 2004; Csomor et al., 2007; Castellano et al., 2007), suggesting that human DCs, like murine DCs, have the capacity to synthesise complement and interact with it. However, several important questions remain, for example, whether moDCs can synthesise a diverse range of complement elements sufficient for local activation and detection of complement effectors; whether pathogen-associated or inflammatory stimuli regulate this synthesis; whether different subsets of DCs, such as dermal DCs (dDCs), Langerhans cells (LCs), plasmacytoid DCs (pDCs) and myeloid DCs (mDCs), have the same characteristics or exhibit different capacities to produce complement compared to moDCs.

In the present study, we addressed these questions by examining the expression of a wide range of complement pathway components, receptors and regulators using distinct subsets of human DCs (moDCs, dDCs, LCs, pDCs, mDCs) and the regulation of complement gene expression in moDCs by inflammatory or pathogen-related stimuli.

2. Materials and methods

2.1. Reagents for preparation of DCs

Microbeads for CD14, CD34, CD1a, and positive selection kits for BDCA-1 and BDCA-4 were purchased from Miltenyi (Surry, UK). GM-CSF, IL-4, IL-1 and TNF-α were purchased from Firstlink (Brierley Hill, UK). SCF and FLT3 ligand were purchased from Biosource Camarilio (CA, USA). TGF-β was purchased from R&D Systems Europe Ltd. (Abingdon, UK). LPS, Prostaglandin E_2 (PGE_2) was purchased from Sigma–Aldrich (Dorset, UK). RNA isolation kit was purchased from Applied Biosystems (Warrington, UK).

2.2. Samples

Collection of human samples (i.e., buffy coats, cord blood, peripheral blood) was approved by the local research ethics committee of Guy's Hospital, London, UK. Human buffy coats were obtained from NBS-South Thames, London, UK. Cord blood was obtained from King's College Hospital, which was approved by the local research ethics committee.

2.3. Preparation of human DCs

Monocyte-derived DCs were generated from peripheral blood monocytes by treatment with GM-CSF and IL-4. PBMC were isolated from buffy coat preparations from healthy donors by Ficoll-Hypaque gradient centrifugation, followed by anti-CD14 bead selection. $CD14^+$ monocytes were cultured in RPMI-10% FCS, 2 mM L-glutamine, and 100 U/ml penicillin and streptomycin in the presence of GM-CSF (20 ng/ml) and IL-4 (20 ng/ml) at 37 °C in 5% CO_2 atmosphere for 5 days. In some experiments $CD14^+$ cells were cultured as described above but in the presence of 10% of human serum (HS) either normal (NS) or heat-inactivated (HIS) instead of FCS. In addition, in some cases moDCs were stimulated at day 5 with LPS (100 ng/ml) for 24 h, or day 4 with TIP (TNF-α, IL-1 both 20 ng/ml and PGE_2 2.5×10^{-6} M) for 48 h. Dermal DCs and LCs were generated from cord blood. $CD34^+$ cells isolated by positive selection were cultured in RPMI supplemented by 10% FCS for 6 days, in the presence of SCF (25 ng/ml), FLT3 ligand (25 ng/ml), TNF-α (5 ng/ml) and GM-CSF (100 ng/ml), and followed by separation of $CD14^+$ and CD14-cells. $CD14^+$ cells (precursor of dDCs) were further cultured for 6 days, in the presence of GM-CSF (50 ng/ml), SCF (25 ng/ml), FLT3 ligand (25 ng/ml), IL-4 (1000 U/ml); while, $CD14^-$ (precursor of LC) were further cultured in the presence of TNF-α (2.5 ng/ml) and TGF-β (5 ng/ml). $CD1a^+$ dDCs and $CD1a^+$ LC were isolated, respectively from the day 12 cultures by positive selection (Rozis et al., 2008). Plasmacytoid DCs were freshly isolated from peripheral blood by CD304 (BDCA-4) positive cell selection. Cells were then further sorted for CD123 (IL-3R) and CD303 (BDCA-2) expression. Sorted cells were then cultured overnight, in the presence of IL-3 (10 ng/ml). Myeloid DCs were freshly generated from peripheral blood by $CD20^+$ depletion, followed by purification of $CD1c^+$ cells using Miltenyi BDCA-1 positive selection kit.

2.4. Conventional RT-PCR

Total RNA was extracted from the cell pellets using Ambion RNA isolation kit and subsequently used for cDNA synthesis. cDNA synthesis was carried out with 5 μg of total RNA, 160 ng of oligo$(dT)_{12-18}$, 500 μM of each dNTP, and 200 U Moloney murine leukemia virus reverse transcriptase in 20 μl of solution (50 mM Tris–HCl pH 8.3, 75 mM KCl, 10 mM DDT, 3 mM $MgCl_2$, 1.5 U/ml RNasin) at 37 °C for 45 min. At the end of the reaction, cDNA was further diluted with sterile water (30 μl of water was added in 20 μl of reaction mixture) and stored at −20 °C until further use. PCR was carried out with 2 μl diluted cDNA (reflecting 0.2 μg of total RNA), 12.5 pmol of each 3′ and 5′ primer pair for each testing gene (the information for primer sequences are shown in supplementary Table) in 25 μl of reaction buffer (Promega, Southampton, UK). The PCR cycle consisted of 1 min at 94 °C, 1 min at 62 °C, and 1 min at 72 °C. Amplified PCR products were visualized after electrophoresis on 1.5% or 2% agarose gel containing ethidium bromide.

2.5. RT-quantitative PCR

RNA extraction and cDNA synthesis were performed as described in Section 2.4. RT-quantitative PCR (RT-qPCR) was performed with an MJ Research PTC-200 Peltier Thermal Cycler and