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Differential expression of functional Fc-receptors and additional immune complex receptors on mouse kidney cells

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ABSTRACT

The precise mechanisms by which circulating immune complexes accumulate in the kidney to form deposits in glomerulonephritis are not well understood. In particular, the role of resident cells within glomeruli of the kidney has been widely debated. Immune complexes have been shown to bind one glomerular cell type (mesangial cells) leading to functional responses such as pro-inflammatory cytokine production. To further assess the presence of functional immunoreceptors on resident glomerular cells, cultured mouse renal epithelial, endothelial, and mesangial cells were treated with heat-aggregated mouse IgG or preformed murine immune complexes. Mesangial and renal endothelial cells were found to bind IgG complexes, whereas glomerular epithelial cell binding was minimal. A blocking antibody for Fc-gamma receptors reduced binding to mesangial cells but not renal endothelial cells, suggesting differential immunoreceptor utilization. RT-PCR and immunostaining based screening of cultured renal endothelial cells showed limited low-level expression of known Fc-receptors and Ig binding proteins. The interaction between mesangial cells and renal endothelial cells and immune complexes resulted in distinct, cell-specific patterns of chemokine and cytokine production. This novel pathway involving renal endothelial cells likely contributes to the predilection of circulating immune complex accumulation within the kidney and to the inflammatory responses that drive kidney injury.

1. Introduction

Immune complex (IC) diseases are an important cause of morbidity and mortality in children and adults. The kidneys are particularly affected by circulating IC. Human kidney biopsy studies and kidney disease registries report IC are present in 45–65% of patients with glomerular disease (Briganti et al., 2001; Swaminathan et al., 2006; U.S. Renal Data System, 2009; Wirta et al., 2007). Circulating IC in systemic lupus erythematosus, IgA nephropathy, Henoch Schönlein purpura, cryoglobulinemia, serum sickness, and chronic infections as well as IC formed *in situ* in membranous nephropathy, lupus nephritis, post-infectious glomerulonephritis, and anti-glomerular basement membrane disease result in immune deposits seen by immunofluorescence staining and electron microscopy. Distribution within the distinct sub-endothelial, sub-epithelial, and mesangial spaces vary amongst these diseases, but the factors determining the pattern of IC deposition within the glomerulus remain debatable. It is also unclear if circulating IC are actively bound or passively trapped within the glomerulus (Alpers et al., 1991). Regardless of the precise mechanism, the kidney appears to be uniquely sensitive to IC deposition compared to other organs.

Previous studies have demonstrated that IC can bind directly to renal parenchymal cells. Radiolabeled aggregates of IgG or IgA localize to the kidney *in vivo* (Barnes et al., 1990; Chen et al., 1988; Gauthier et al., 1982; Mauer et al., 1972; McCluskey et al., 1960) and bind to mesangial cells (MCs) *in vitro* with high specificity (Bagheri et al., 1997). IC binding leads to complex internalization, cellular proliferation, and release of inflammatory cytokines MCP-1 and IL-12, in rat (Gomez-Guerrero et al., 1994; Sedor et al., 1987; Singhal et al., 1990), human (Radeke et al., 1994), and mouse MCs (Radeke et al., 2002). Visceral epithelial cells isolated from human glomeruli (also referred to as podocytes) have also been shown to bind IC (Haymann et al., 2004). Despite the *in vivo* findings in animal models that circulating IC often first form deposits in subendothelial

Abbreviations: BMDCs, bone marrow-derived cells; EnCs, endothelial cells; FcRs, Fc-receptors; HA-mIgG, heat aggregated mouse gamma globulin; IC, immune complexes; MCs, mesangial cells; PAP-IC, peroxidase-anti-peroxidase immune complexes; REnCs, renal endothelial cells; SMA, smooth muscle actin; TfR, transferrin receptor.

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locations (Barnes et al., 1990; Gauthier et al., 1982; Mauer et al., 1972; McCluskey et al., 1960), the capacity of renal endothelial cells (REnCs) to bind IC has not been previously described.

Bone marrow-derived cells (BMDC) express receptors for IC that bind to the Fc-region of Immunoglobulin molecules and have thus been named Fc-receptors (FcR). FcR are responsible for mediating antibody-dependent cell cytotoxicity by neutrophils and NK cells, antibody-mediated cell phagocytosis by macrophages, and clearance of circulating IC and antibody bound cells (Ravetch and Bolland, 2001). In addition, there are Ig transporters and FcR-like proteins that can also bind IgG (Wilson et al., 2012). Historically, the expression of FcR by resident glomerular cells has been extremely controversial. There is a paucity of data from human biopsy studies (Jennette et al., 2006), but numerous reports demonstrate expression by cultured MCs. Constitutive expression of FcγRIII protein and RNA has been reported in rat (Santiago et al., 1989) and human MCs (Morcos et al., 1994). Stimulating FcγR-specific antibodies activate the same pathways in rat MCs as those activated by preformed IC (Morcos et al., 1994; Radeke et al., 1994; Santiago et al., 1989), and receptor binding induces cytokine production (Morcos et al., 1994). Other groups have failed to show basal expression of FcγRs (Matre et al., 1980), although expression could be induced with IFNγ and LPS (Radeke et al., 1994; Uciechowski et al., 1998). Mouse MC constitutively express FcγRIIb, but FcγRIII expression required stimulation with IFNγ (Radeke et al., 2002).

In order to better explain the physiologic and pathologic responses to circulating immune complexes in the kidney, model IC derived from mouse IgG were tested in primary cultures for each resident glomerular mouse cell type. In addition, differences in functional cellular responses were examined to identify potential cell-specific mechanisms that could contribute to renal injury.

2. Results

2.1. Glomerular cells binding to immune complexes

Primary mouse MCs were treated with either heat aggregated mouse immunoglobulin (HA-mIgG) or preformed antigen-antibody IC. HA-IgG has been utilized in the past as a model for studying cellular responses to IC, but previous publications used only human HA-IgG. Mouse MC stain positively with HA-mIgG, but much more weakly with equimolar concentrations of monomeric mouse IgG (Fig. 1). Staining by direct immunofluorescence using labeled reagents was similar to staining by indirect immunofluorescence using unlabeled reagents followed by a labeled anti-mouse IgG secondary $F(ab')_2$ fragments. There was no staining of mouse MCs treated with secondary antibodies alone. Preformed antigen-antibody IC composed of peroxidase and mouse anti-peroxidase antibodies (PAP-IC) also stained mouse MCs. Staining occurred in the presence or absence of FBS.

The staining of mouse podocytes with HA-mIgG, PAP-IC, and monomeric IgG was no greater than that seen using secondary antibodies alone (Fig. 1E–H). Primary mouse podocytes were tested, as was a conditionally immortalized podocyte cell line. Staining was also not detected on mouse REnCs treated with monomeric mouse IgG or secondary antibodies alone. However, treatment of REnCs with HA-mIgG and PAP-IC resulted in staining comparable to that seen in mouse MCs (Fig. 1I–L).

Previous studies have implicated low affinity FcγRs in mediating the binding of HA-IgG to MCs (Morcos et al., 1994; Radeke et al., 1994, 2002; Santiago et al., 1989). To assess the role of FcγRs in mediating mouse MC staining by HA-mIgG and PAP-IC, we used the rat blocking Ab, clone 2.4G2. Pre-incubation of mouse MC with 2.4G2 greatly reduced, but did not eliminate staining with HA-mIgG or PAP-IC (Fig. 2A–D). In contrast, staining of mouse REnCs was unchanged after pre-incubation with inhibitor 2.4G2, suggesting that staining was independent of FcγRs (Fig. 2E–H).

To further investigate the staining of REnCs, trypsinized cells in suspension were treated with HA-mIgG or PAP-IC and assessed by flow cytometry. Staining of REnCs could be detected consistently in cells in suspension in the absence or presence of Fc-receptor inhibitor 2.4G2 (Fig. 3). Similar results were seen with directly conjugated IC or with indirect immunofluorescence using unlabeled IC and labeled anti-mouse IgG secondary $F(ab')_2$ fragments. The immortalized mouse macrophage cell line, RAW264, which is known to express high levels of Fc-receptors, stained with HA-mIgG and PAP-IC by immunofluorescence microscopy and by flow (MFI 364 ± 10 and 854 ± 41). In comparison, the MFI of HA-mIgG and PAP-IC treated and unstained REnCs were 172 ± 24, 165 ± 79, and 11 ± 2.

The immortalized mouse brain endothelial cell line, bEND3, was tested to assess the generalizability of REnC staining with labeled HA-mIgG or PAP-IC. By immunofluorescence microscopy, the staining of bEND3 cells was no greater than that seen using secondary antibodies alone (data not shown). By flow cytometry, <2% of trypsinized bEND3 cells in suspension could be found to bind HA-mIgG (Fig. 3). The MFIs of HA-mIgG and PAP-IC treated and unstained bEND3 cells were 17 ± 11, 14 ± 9, and 13 ± 5. There was no effect of blocking antibody 2.4G2 on staining. Therefore, HA-mIgG and PAP-IC appear to preferentially bind to REnCs over brain EnCs.

2.2. Cell-specific glomerular expression of Fc-receptors and immunoglobulin transporters

In mice, there are seven FcR (encoded by one gamma, one beta, and seven alpha chain genes), six FcγR-like genes, and three Ig transporter genes (pIgR, Fcgrt, and Fcgbp). To determine which IC receptors were expressed on mouse glomerular cells, a panel of primers was developed and expression was assessed by RT-PCR (Fig. 4).

Of the transcripts tested, mouse MC expressed Fc-receptor transcripts Fcgr3, Fcgr4, and Fcerg1. MC also expressed the FcR-like transcript Fcrl5. Podocytes expressed Fcrl1 and the IgG transporter Fcgrt. REnCs expressed Fc-receptors Fcgr2b, Fcgr3, Fcerg1, Fceb1, and Fcamr. These patterns would allow for the production of discrete subsets of IC receptors on the three main cell types of the renal glomerulus. By qRT-PCR, the absolute mRNA expression levels were shown to be low. In 3 independent MC cultures, mRNA expression for Fcgr3, Fcgr4, and Fcerg1 were 100- to 1000-fold lower than levels of GAPDH expression. In 3 REnC cultures, mRNA expression for Fcgr2, Fcgr3, Fcerg1, Fceb1, and Fcamr were measurable, but >10,000-fold lower than the levels of GAPDH expression.

Consistent with the low mRNA levels, significant expression of Fc-receptors could not be reliably detected by immunostaining REnCs or bEND3 cells on culture slides, or by staining mouse kidney sections (data not shown). By flow cytometry, there were no differences in the expression of FcR proteins between REnCs and bEND3 cells. Only a small degree of fluorescent staining was noted with antibodies for FcγRIV and TfR (mean ΔMFI *vs.* control Abs were 17.7 ± 4.6 and 20.7 ± 6.4 for REnCs, compared to 9.2 ± 1.6 and 28.5 ± 2.1 for bEND3 and 12.7 ± 5.1 and 194.1 ± 34.4 for RAW264) (Fig. 5). Antibodies for FcγRI, FcγRIIb, FcγRIII, the common γ-chain (FcR-gamma), and transferrin receptor (TfR) detected significant expression on RAW264 macrophages, which were used as a positive control. There were no known IC receptors with preferential expression on REnCs compared with bEND3 cells. Therefore, REnCs are likely to bind and respond to IC binding in a novel manner, independent of Fc-receptors and known immunoglobulin binding proteins.