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A novel P2X7 knockout rat is not protected from experimental glomerulonephritis or vasculitis

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Introduction

P2X7 is an ionotropic receptor activated by extracellular ATP which is expressed on immune cells including macrophages, dendritic cells and lymphocytes and is up-regulated on non-immune cells following injury. P2X7 has previously been shown to be important in inflammation and fibrosis, and may also play a role in autoimmunity. However, recent studies have shown both strains of P2X7 deficient mice are incomplete knockouts and have generated conflicting results. Therefore, we have assessed the importance of P2X7 in autoimmunity and glomerulonephritis (GN) using a novel P2X7 knockout (KO) rat.

Methods

A novel P2X7 knockout (KO) rat was created using zinc finger nuclease technology which resulted in a 2 base pair insertion in exon 10. Cells and tissues were used for extraction of protein and mRNA for Western blot and PCR. Bone marrow derived macrophages (BMDM) and dendritic cells (BMDC) were used to assess P2X7 function using uptake of Yo-pro-1 fluorescent dye in response to ATP and by stimulating cells with LPS 1μg/ml followed by ATP 5mM and measuring IL-1β production. Three in vivo models, nephrotoxic nephritis (NTN), experimental autoimmune GN (EAG) and experimental autoimmune vasculitis (EAV) were used to induce disease in P2X7 KO and WKY WT rats. Disease severity and development of autoimmunity were assessed in each model (NTN- Day 28, EAG- day 28, EAV- day 42).

Results

No P2X7 protein was detected in cells and tissues from the P2X7 KO rat (Figure 1a). BMDM and BMDC from the WKY WT but not the P2X7 KO rat formed large pores and took up Yo-pro-1 in response to stimulation with ATP (Figure 1b). As expected WKY WT BMDM produced significant IL-1β when stimulated with LPS plus ATP compared to cells stimulated with LPS alone. BMDM from P2X7 deficient rats did not produce significant amounts of IL-1β (Figure 1c). Both WKY WT and P2X7 KO BMDC are able to cleave IL-1β in response to stimulation with LPS alone via pathways that are independent of P2X7 and K+ efflux from cells but dependent on capsase-1 (Figure 1d).

P2X7 KO rats were not protected from disease in the NTN, EAG or EAV models. P2X7 KO and WKY WT rats had similar renal function, degree of urinary abnormalities, histological disease severity, lung injury and circulating and deposited antibody. Results from a representative EAV experiment are shown in Figure 1e.

Conclusions

This is the first description of a novel P2X7 KO rat showing it is a true knockout. BMDC (but not BMDM) from both WKY WT and P2X7 KO rats do not require a second signal via P2X7/ATP in order to produce active IL-1β. Given that P2X7 KO rats were not protected from experimental GN it may be that cell types which are

able to produce cytokine by P2X7 independent pathways play a relatively greater role than those which cannot utilise this mechanism.