Immune complexes recruit and activate proinflammatory human slanDC in lupus nephritis

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INTRODUCTION

Lupus nephritis is a serious complication of systemic lupus erythematosus. Among the different forms of lupus nephritis IC deposition to renal glomeruli is a hallmark of its most severe manifestation called proliferative lupus nephritis. SlanDCs are highly proinflammatory human blood dendritic cells (DCs) that serve as a major and early source of IL-12 (1) and TNF- α (2). Within tissue slanDCs have been identified as inflammatory dermal DCs in psoriasis (3) and lupus erythematosus (4). SlanDCs stand out by their high-level expression of the low-affinity Fc γ receptor III (CD16), a receptor for mostly complexed immunoglobulin, and display an outstanding capacity to bind small immune complexes (5). We here show that immune complexes recruit slanDCs via CD16 in lupus nephritis.

SUMMARY

1. SlanDCs are found in glomeruli from lupus nephritis patients with immune complexes deposition (class III and IV) and produce locally TNF- α .
2. Glomerular deposition of immune complexes in immunodeficient NSG mice mediates recruitment of human slanDCs and CD16-transfected Jurkat cells
3. SlanDCs (but not CD1c⁺ DCs or pDCs) adhere to immobilized immune complexes under conditions that match venous blood flow (> 0.5 dynes/cm²)
4. Adherence of slanDCs to immune complexes is largely CD16-dependent.
5. Antibody deposition on human endothelial cells mediates a CD16-dependent recruitment of slanDCs from the flow.
6. slanDCs engaged with immobilized immune complexes became activated and produce the neutrophil recruiting chemokine CXCL2. These slanDCs also produced TNF- α which in endothelial cells induce the production of the fractalkine (CX3CL1).

CONCLUSION

slanDCs are a new proinflammatory cell type in lupus nephritis with potential relevance for the early steps in the disease pathogenesis. Moreover, expression of CD16 on slanDCs contributes to the binding to immobilized immune complexes (ICs) which may orchestrate the early IC induced inflammatory response in glomeruli.

1) Schäkel et al, *Immunity*, 2006; 2) Schäkel et al, *Immunity*, 2002; 3) Hänsel and Günther et al, *JACI*, 2011; 4) Hänsel et al, *Journal of Autoimmunity*, 2013; 5) Döbel et al, *Blood*, 2013

RESULTS

1. slanDCs outstandingly bind small soluble immune complexes (IC) and accumulate in lupus nephritis

2. Immune complexes recruit slanDCs in vivo

Figure 2 – (A) Schematic representation of the in vivo mouse model. Percent of glomeruli with slanDC (B) or Jurkat cells transfected or non-transfected (C) recruited by IC.

3. Efficient capturing of flowing slanDCs by immobilized ICs

Figure 3 – (A) Experimental setup of the flow chamber adhesion assay with adjustable shear stress (B) Adherence of slanDCs to immobilized ICs under physiologically relevant fluid shear stress (0,5 dynes/cm², N = 4) (C) Representative examples of the same experiment after different timepoints

4. CD16-dependent slanDC recruitment by immobilized ICs

Figure 4 - Receptor-specific adherence of slanDCs to immobilized ICs. Receptor specificity was achieved by using specific blocking mAbs for CD16 and CD32 or IVIG.

4. Antibody-mediated arrest of slanDCs on endothelial cells

Figure 4 – (A) Recruitment of slanDCs to endothelial cells (HDMECs) at physiologic shear stress after coating of endothelial cells with anti-CD105 (0,5 dynes/cm², N = 3) (B) Representative examples (t = 30 min)

5. Immobilized ICs stimulate slanDCs to induce an inflammatory immune response

Figure 5 – (A) Single-color pictures and combined colors for the detection of TNF- α (green), slanDCs (red) and cell nuclei (DAPI; blue) in lupus nephritis glomeruli class III. Original magnification $\times 200$. (B) TNF α , IL-6 and CXCL2 protein levels induction by immobilized immune in the slanDC culture medium were determined by ELISA. (C) TNF α and IL-6 levels were also measured in the culture medium from CD1cDC and monocytes incubated or not with IC. (D) HDMECs were cultured for 20h with supernatants from slanDC previously incubated with immobilized ICs in the presence or not of a TNF- α mAb inhibitor (10 μ g/ml). CX3CL1 protein levels.