ImmunoTools *special* Award 2014

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Interaction between plasmacytoid DC and renal epithelial cells; at the crossroad of allo- and anti-viral-immunity

Patients who have been transplanted with a donor kidney are on lifelong immune suppression, in order to prevent graft rejection. Although this clearly benefits graft survival, the downside being that they are susceptible to infections, of which cytomegalovirus (CMV) and BK virus are most commonly seen. As leukocytes specialized in aiding the clearance of viral infection, plasmacytoid dendritic cells (pDC) can produce vast amounts of interferon-α (IFN-α) upon viral recognition through Toll-like receptor (TLR)-7 or TLR-9. We have previously demonstrated that pDC are present at very low numbers at the time of transplantation, but show a remarkable increase in the tubulointerstitium of human renal biopsies with acute rejection. Moreover, viral infections are associated with the initiation of acute rejection, and since pDC are present at high numbers during rejection, they can interact locally with viral infected cells. These data combined can be indicative of a role of pDC during rejection, however, very little is known on the role of this DC subset in graft rejection. In recent years pDC have been characterized as antigen presenting cells (APC), capable of phagocytosis and priming antigen-specific $CD4^+$ and $CD8^+$ T cells.

In order to gain more insight in the interaction of pDC with renal cells, we performed co-culture experiments, showing that viable, apoptotic or necrotic human renal epithelial HK2 cells affected pDC phenotype and IFN-α production only marginally. As expected, the addition of the viral DNA mimic CpG led to pDC activation. The additional presence of either apoptotic or necrotic HK-2 cells did not affect pDC phenotype or their IFN-α production. Interestingly, co-culturing pDC with viable HK-2 in the presence of CpG significantly increased their expression of co-stimulatory molecules, and the production of IFN-α. In contrast, the expression of the co-

inhibitory molecule PD-L1 was 2.5-fold diminished, compared to co-culturing with necrotic cells and pDC alone. Of importance, pre-treatment of HK2 cells with CpG was sufficient to strongly activate pDC, equivalent to direct CpG-mediated pDC activation. We have reason to believe this effect is mediated by a soluble factor, and is in part mediated via a contact-dependent mechanism.

To gain more insight in the interactions of epithelial cells and pDC, we would like to perform a full phenotypic analysis of both pDC and HK2 cell-associated antigens that are altered or could be involved in mediating the observed effects, such as CD2, CD4, CD45RA, and CD56, for which the ImmunoTools toolbox would be of great help. We would like to analyze the soluble factors produced by HK2 cells following CpG stimulation and viral infection, and plan to visualize the interactions between HK-2 cells and pDC by means of (confocal) microscopy (e.g. CD45, HLA-ABC).

Moreover, we will assess the uptake of apoptotic, necrotic and virus-infected cells, and the subsequent effect on pDC function. To this end, we will be looking at pDC phenotype (e.g. CD80, CD86, CD40, HLA-DR), the production of cytokines e.g. (IL-4, IL-6, IL-12p40, TNFa ELISA sets), their migratory capacity (MIP3) and their ability to induce T cell proliferation and activation (CD3, CD4, CD8, CD25, CD69). Moreover, we will analyze the effect of pDC interactions with (infected) HK2 cells, and how this will affect T cell memory status (e.g. CD3, CD4, CD8, CD27, CD62L), T cell cytokine production, and pDC capacity to perform indirect- and cross-presentation of antigen (IFN-γ).

ImmunoTools *special* AWARD for **Jurjen M. Ruben** includes 25 reagents

FITC - conjugated anti-human CD40, CD45RA, CD86, HLA-ABC, HLA-DR,

PE - conjugated anti-human CD2, CD3, CD11b, CD27, CD45, CD80, IFN-gamma,

PerCP - conjugated anti-human CD8,

APC - conjugated anti-human CD4, CD25, CD40, CD56, CD62L,

human IL-4 ELISA-set for 96 wells, human IL-6 ELISA-set for 96 wells, human IL-12p40 ELISA-set for 96 wells, human TNFa ELISA-set for 96 wells (each 3 reagents),

recombinant human cytokines: MIP3 DETAILS more AWARDS