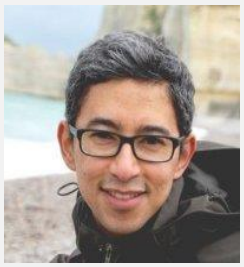
ImmunoTools *multiplex* Award 2014



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Prognostic markers for Graft-versus-host disease

Graft-versus-host disease (GVHD) in 30-60% of patients and has a ± 50% mortality rate after hematopoietic stem cell transplantations (HSCT), which is often used as cancer therapy (*Barton-Burke, M. et al 2008, Robin, M et al 2009*). HSCT, while beneficial in restoring components of a depleted host immune system, can also be detrimental to the host when the transferred T cells are activated by minor or major histocompatibility antigens of the host (*Antin, J.H. et al 1992*). These T cells expand and mount a strong immune response against the host, including cytokine production and direct attack of the host tissue, which results in organ damage, sepsis and morbidity or mortality.

We discovered that IL-27 is produced during murine acute GVHD (*Marillier, R.G. et al. 2014).* IL-27, a member of the IL-6 and IL-12 family is a pleiotropic cytokine, being at the same time an initiator of Th1 type immune reactions (*Pflanz, S. et al. 2002*) and a barrier against excessive inflammation (*Yoshida, H. et al. 2009*). IL-27 is formed by non-covalently linked p28 (also called IL-30) and Epstein-Barr virus-induced gene 3 product (EBI3), which are structurally related to IL-12p35 and IL-12p40, respectively (*Lucas, S. et al. 2004*). IL-27 binds to a heterodimeric receptor, comprising WSX-1/IL-27Rα and gp130, which is expressed on hematopoietic stem cells, all lymphoid and myeloid cells, vascular endothelium and keratinocytes (*Pflanz, S. et al. 2004*).

IL-27 is produced by antigen presenting cells in response to bacterial products or inflammatory mediators and orients naïve $CD4^+$ T cells to the Th1 pathway by inducing expression of IL-12Rβ2 (*Yoshida, H. et al. 2001*), which then enables IL-12 to stimulate IFN-γ production. It also triggers $CD8^+$ T cell maturation into fully differentiated cytolytic T lymphocytes (*Morishima, N. et al. 2005*) and stimulates IgG2a subclass switching (Kamiya, S. 2004). As it inhibits Th2, Th17 and Th9 cytokine production, IL-27 plays a key role in Th1 response skewing.

Short-term treatment with a mouse anti-mouse anti-IL-27 monoclonal antibody (Uyttenhove, C. *et al.* 2011) lead to protection from aGVHD, that was permanent and was accompanied by a down regulation of IFNγ production, CTL activity, an upregulation of Th2-type immunoglobulin levels and an increase in Foxp3 positive T regs (*Marillier, R.G. et al. 2014*). There is a persistent effort to employ immune-therapy in GVHD. However the treatments that have been tried in the clinic have had limited success as attested by the remaining mortality rates. Our observation suggests that IL-27 could be a novel target for GVHD prevention.

The next step for us now, is determined if these findings are relevant for humans. Firstly is IL-27 and its family of cytokines induced during GVHD in humans? If so, secondly, at what

time point is IL-27 induced? Thirdly is the IL-27 signature, associated with disease out come? Lastly besides our interest in IL-27, there are no clearly defined prognostic markers of acute or chronic GVHD. To answer these questions we have collected serum and cells from 100 leukemia patients before and several time points after HSCT for screening of biomarkers.

Proteins are thought to be more representative than other cellular metabolites, to the ongoing pathophysiology of a disease (*Paczesny, S. Current Biomarker Findings 2012*) and therefore we would like to measure proteins in the serum of these patient samples.

In serum, the quantities of cytokines and proteins, even when induced, maybe at a very low concentrations and thus poorly detectable by ELISA. In addition, ELISAs also require large sample volumes and for the purpose of screening for several molecules, are time consuming and expensive.

Multiplex would greatly help us quickly determine which cytokine, chemokines and other proteins are present in the patients that have received HSCT and develop GVHD or not. These results may reveal much needed biomarkers to predict GVHD and also new therapeutic targets for GVHD.