ImmunoTools *multiplex* Award 2014

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Characterisation of the leukemia supporting macrophage secretory phenotype

Chronic Lymphocytic Leukemia (CLL) is monoclonal B-cell disorder that is characterised by an accumulation of leukemic cells in the peripheral blood (PB), bone marrow (BM) and lymph nodes (LN). The inevitable chemo-resistance that develops after treatment of CLL with conventional chemotherapeutics, is considered to be the result of either CLL cell intrinsic mutations or interactions of the CLL cell with the LN microenvironment, where interactions take place with, among others, T-cells and macrophages.

Within our group, we study the effects of these last two interactions, where my project mainly focuses on the interaction with macrophages. Our main aims are to identify the signals causing the macrophage survival effect, and characterise the molecular and functional events in CLL cells following macrophage stimulation, including intracellular signalling pathways.

We have previously shown that indeed both activated T-cells and monocyte derived M1/M2 differentiated macrophages in vitro have a supportive function for the CLL cells, conferring chemo-resistance (T-cell) or a survival advantage (T-cell and macrophage).

Using a co-culture system with either autologous T-cells or CD40L (a T-cell derived co-stimulus) overexpressing NIH-3T3 mouse fibroblasts we were able to mimic the chemo-resistance of CLL cells in vitro. Microarray studies of CLL cells comparing both stimuli have furthermore shown that the transcriptional profile after both stimuli is largely comparable, designating CD40L as the main responsible factor for the chemo-resistance conferred by T-cells (*Pascutti et al, Blood. 2013 Oct 24;122(17):3010-9*).

In contrast, the vast array of cytokines that macrophages are able to produce, which is furthermore dependent on their differentiation, makes it difficult to pinpoint the responsible factors for the macrophages' survival benefit conferred to CLL cells. Furthermore, an increasing set of literature is becoming available showing tumor cells creating their own supportive microenvironment, by secreting factors that differentiate neighbouring cells to support their survival, indicating the need to study both monocyte→tumor and tumor→monocyte interactions.

In this light Bögels et al (*Oncoimmunology. 2012 Sep 1;1(6):798-809*) for instance show that supernatant derived from different tumor types dictates the skewing of monocytes resulting in either an anti- or pro-tumor differentiated macrophage. Also, preliminary data from our lab suggest a similar skewing effect of monocytes when differentiated with serum from CLL patients (compared to healty donor serum).

With this in mind, our study will focus on unravelling the reciprocal interaction between macrophages and CLL cells.

To this end, we will use monocytes derived from healthy donors and differentiate them to an M1/M2 differentiated phenotype before co-culturing them with CLL cells for at least N=5 CLL samples. After 72h, supernatants will be collected to be analysed with the multiplex array. Furthermore RNA expression profiling of both the macrophages and CLL cells will be performed using microarrays.

Likewise, we plan to identify the effects of CLL cells on monocyte differentiation by differentiating monocytes using serum from our CLL serum database and analyse the collected supernatant with the multiplex array.

The multiplex array will allow us to investigate in detail the pro-survival skewing of the monocytes by CLL cells and create an unbiased overview of cytokines secreted by monocyte derived cells that support CLL cell survival. This knowledge can then be used to intervene with cytokines on different levels to nullify the survival effect that macrophages confer to CLL cells, possibly having therapeutic implications.