Report of J. Gommerman project performed in PICT-curie (Paris Centre Node)

Project title: Effect of vitamin A on splenic dendritic cells subset localization.

Scientific background:

Dendritic cells (DCs) are professional antigen presenting cells which are widely distributed throughout the body and are able to capture and present antigens to orchestrate an appropriate immune response. Several subsets of DCs have been distinguished based on their immunological function, their phenotypic markers and their localization. In the spleen, conventional DCs (cDCs) can be divided into three subsets based on the expression of the co-receptors CD4 and CD8. The $CD8^+CD4^-$ cells are specialized for cross-presenting antigen to trigger CD8+ T cell responses, whereas $CD4^+$ and double negative $CD4^-CD8^-$ DC can activate CD4+ helper T cells. The differentiation and homeostasis of splenic DCs is tightly regulated by several factors that influence both the composition and the appropriate positioning of DC. For example, splenic $CD4^+$ cDCs need to localize within the marginal zone bridging channels (MZ BC), a restricted area where the T cell zone is connected to the red pulp and where cDCs can take up antigens coming from the blood.

The factors that allow the appropriate localization of $CD4^+$ DCs remain uncharacterized. It has been reported that the G-protein coupled receptor EBI2 and its main ligand 7α,25-OHC were required for the localization of cDCs in the bridging channels as well as signals mediated by the TNF superfamily LTβR. In the case of LTβR signaling, its ligand LTα1β2 is derived from B cells in order to maintain $CD4^+$ splenic DCs. Recently, several studies have pointed to the importance of retinoic acid (RA), a metabolite of vitamin A, in the control of $CD8^-CD4^+CD11b^+$ splenic DCs. So far, the exact mechanisms of action of RA in maintaining the composition of splenic DCs is unknown, although one possible explanation of the observed RA effects could be that RA may modulate the phenotypic composition of spleen-resident DCs by influencing their localization within the splenic white pulp.

Aim: Investigating the effect of vitamin A on splenic DC localization.

Methods : Tissue sections derived from mice treated with different vitamin A diets were generated and immunostained with antibodies for specific markers to stain distinct areas of the spleen and different subsets of DCs in the lab of Dr J. Gommerman at the University of Toronto. These tissue sections were subsequently examined in the imaging facility of the Curie Institute in Paris, a Member of the INBS France-BioImaging. We tested different confocal microscope (scanning and spinning disk) with low magnification, to image a large field of view in order to overview the organization of the cells in thick tissue sections in 3 dimensions.

Results :

1- Effect of vitamin A diet on the localization of CD11b+ CD8- ESAM+ DCs

To investigate the localization of the $CD4^+CD11b^+CD8^-ESAM^+$ DC subset, we took advantage of the expression of the C-type lectin DCIR2 which is expressed by all $CD4^+$ DCs and which can be detected with the 33D1 antibody. The precise positioning of this subset was characterized by staining distinct areas of the spleen: the marginal zone (anti-CD169 antibody), the B cell follicle area (anti-IgD antibody) and the T cell zone (anti-TCR-beta). Acquisitions were performed on a confocal microscope LEICA SP8. The spectral detection

system of this microscope allowed us to separate well the emission signals from the 3 different fluorophores.

The observations of the control spleen sections revealed that the 33D1+ DCs are mainly located within the MZ BC as has been previously described. Interestingly, when mice were under a vitamin A deficient diet, the overall 33D1 staining was dramatically reduced. We could detect some of the remaining 33D1+ cells in the MZ BC area but most of the MZ BC were negative for 33D1 in the VAD spleen sections.

2- Detection of collagen in the marginal zone bridging channel

Since the accumulation of 33D1+ DC occurs in a very specific area of the spleen (MZ BC), we wondered if splenic matrix components (stroma) could be involved to support this accumulation. To answer this question, we used a 2-photon microscope to detect collagen fibers by Second Harmonic Generation (SHG) in the different tissue sections. We used a Nikon A1R-MultiPhoton upright microscope coupled with an Insight Deepsee laser (Spectra-Physics) tunable oscillator (600-1300nm). Three independent detectors GaAsP acquire in parallel two fluorescence signals and the Second Harmonique Generation (SHG) signal emitted by the fibrillary collagen type I.

We detected accumulation of collagen in the MZ BC of the control spleen sections with fibers entering the T cell zone. This observation is in agreement with a previous report describing a network of follicular reticular cells connecting the T cell zone with the marginal zone. The same experiment performed with VAD spleen sections revealed reduced amount of collagen within the MZ BC which could explain, at least indirectly, the decrease of 33D1+ cells in this area when mice are VAD.

Contributions :
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