The Moran Foundation

Progress Report

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Title:

Transcriptional control of lymphoid proliferation and development

(The project is still active)

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Transcriptional control of lymphoid proliferation and development

We focused on the study of the protein MEF (also known as ELF4), a member of the ETS-family of transcription factors (TF). We have previously described the role of this TF in the development and function of natural killer cells by regulating the expression of perforin gene (Lacorazza et al. Immunity, 2002). MEF also participates in the maintenance of hematopoietic stem cells in the bone marrow by controlling the entrance to the cell cycle (Lacorazza et al., submitted). In this proposal we started new aspects of the project: 1)- Development of natural killer cells in the bone marrow and 2)- Study lymphoid proliferation in the absence of MEF expression.

A. Specific Aims

Aim 1. *Define the role of MEF in the control of lymphoid proliferation and the involvement in lymphoproliferative diseases.* Generate a mouse model of in vivo T cell activation in the absence of MEF

Aim 2. *Define the spatial localization of early NK cell precursors in the bone marrow and how these are perturbed in MEF-deficient mice.* Identify lodging sites of purified progenitors in the bone marrow of wild type and MEF-deficient mice.

B. Studies and Results

Aim 1. MEF is a cell cycle regulated protein and its loss leads to augmented lymphoid proliferation upon cellular activation (in vitro). We therefore decided to develop a mouse model of T-cell activation in the absence of MEF expression. We crossed a MEF -/- male with an OT-1 TCR transgenic female. OT-1 transgenic mice express a TCR specific to $OVA^{257-264}$ peptide in the context of H2-Kb (strain C57BL/6-Tg[TcraTcrb] 1100Mjb/J, Jackson Laboratory). The first generation (F1) was screened for MEF gene, neo gene, and OT-1 transgene; heterozygous females positive for OT-1 transgene were chosen to continue the cross to a male knockout. 25% of mice in the second generation (F2) are MEF -/-, $OT\text{-}1^{+}$. This part of the project is slow, inherent to the mouse breeding, although straightforward.

While we were advancing in the breeding of our mouse colony, we worked on the optimization of adoptive transfer of CFSE-labeled CD8 positive cells and in vivo activation by injecting the cognate peptide. This aspect of the project proved to be more complicated that we initially expected. We tested different purification systems for CD8

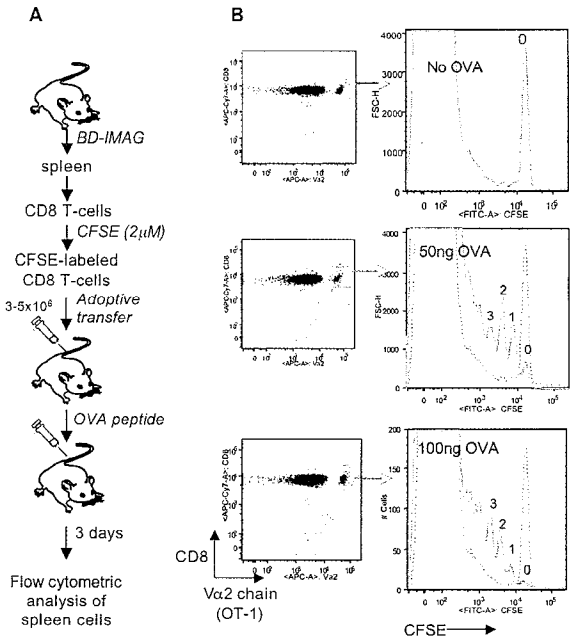

Figure 1: Developed model of in vivo T-cell proliferation to be used then with $OT\text{-}1^{+}$, MEF -/- mice. A- Diagram of the procedure that comprised transgenic T-cell purification, labeling, adoptive transfer, injection of OVA, analysis of the proliferation of donor cells (CFSE cell tracking). B- After 3 days of in vivo activation and proliferation, splenocytes were analyzed for the presence of CFSE positive cells. Note: w/o OVA there is no division, plus OVA cells divided in a dose response manner.

positive cells, different concentrations of CFSE to increase detection capacity with low cytoxicity, different concentrations of OVA peptide to activate the transgenic TCR in vivo, and finally flow cytometric detection of CFSE-labeled transplanted cells. Of note, CFSE labeling was used as cell tracking system and also to measure cell division, as the fluorescence intensity decreases after each division. In the optimized protocol (Figure 1), we used the BD-IMAG system to purified CD8 T-cells by negative selection (to avoid activation due to antibody crosslink) from the spleen of OT-1 transgenic mice (used for the optimization). CD8 positive splenocytes were then labeled with the cell tracking dye CFSE (2 μM) and immediately transferred to non-irradiated recipient mouse (by intravenous injection). After 24 hours, OVA peptide was injected intravenously at different concentrations (0, 50, 100, 200, 500, 1000 ng). The selection of optimal OVA peptide concentration is key as excessive antigen will lead to massive proliferation and apoptosis and low concentration will minimally activate specific T cells. We are now in the process of carrying out this procedure with OT-1 transgenic mice that are also MEF-null.

Aim2. We began studying NK cell development from primitive hematopoietic progenitors in the bone marrow. NK cell development is divided into two developmental processes: 1)- generation of NK progenitors (NKP) from hematopoietic stem cells (HSCs) via the action of stem cell factor (SCF), Flt3 ligand, and IL-7, and 2)- IL-15 dependent maturation of NKP. We isolated from the bone marrow of C57BL/6 mice cells that do not express lineage markers (called lineage negative), eliminating all mature blood cells. Putative NKP were then purified by cell sorting based on the expression of NK1.1 (NK cell marker) and c-kit (marker of early progenitors). NKP should be negative for the cell surface expression of NK1.1 as this is a marker of mature NK cells. We identified as NKP the lineage negative that are NK1.1$^-$ c-kit$^+$ as they converted into c-kit negative after in vitro culture in the presence of SCF, Flt3L and IL-7 (step 1) and then acquired markers of mature NK cells (CD94, NK1.1, and 2B4) by incubation with IL-15 (step 2) (Figure 2). We are now moving into the purification of this cell subset and labeling with CFSE and transplanted into mice to follow the engraftment in the bone marrow as we described in our proposal. We will continue studying this to hopefully publish in a year.

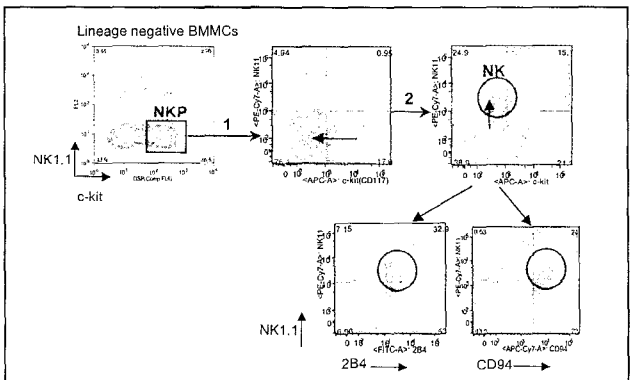

Figure 2: NK cell development in vitro from bone marrow NK-progenitors (NKP). This method is divided into two incubations: (1) SCF, Flt3L, IL7 for one week, (2) IL-15 for one week. At the end of this procedure the culture is predominantly mature NK cells (NK1.1$^+$, 2B4$^+$, CD94$^+$).

C. Significance

This study will contribute to the general knowledge of the transcriptional control of lymphoid cells: T and NK cells, important for adaptive and innate immunity. We will use both models to study the effects of myeloablative treatments, such as chemotherapy and radiation, aiming to find ways to improve immunological recovery.

D. Publications

We have not published yet this work. We expect to test now both models and be able to submit a manuscript next yea