IRREVERSIBLE MARKING OF DENDRITIC CELLS IN VIVO

For Contributed Volumes

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1. INTRODUCTION

Dendritic cells (DC) are bone marrow derived antigen presenting cells that function as sentinels of immune system (Akbari et al., 1999; Bancherea, 2000; Banchereau and Steinman, 1998; Cutler et al., 2001; Mellman and Steinman, 2001). In the peripheral tissues such as skin, they exist as immature DC - cells that are highly efficient at antigen uptake via receptor mediated and non-receptor mediated mechanisms. Upon activation by tissue damage, CpG DNA, pathogens or inflammatory cytokines the immature DC transform into mature DC, a cell type optimized for antigen presentation and T cell activation. DC efficiently process the ingested antigens and present the resultant peptides on the cell surface in the context of class I and class II MHC. Mature DC up regulate expression of class II MHC and activation markers such as CD54, CD58, CD80 and CD86. Mature DC down regulate chemokine receptors like CCR1, CCR5, and CCR6 that keep them in the non-lymphoid tissues and up regulate expression of CCR7, which enables the them to migrate to the draining lymph nodes (LN). In the draining lymph nodes, the antigen bearing mature DC move to T cell area and activate quiescent naïve CD4 and CD8 T cells; DC are the most potent initiators of primary immune responses - they are two orders of magnitude more effective as antigen presenting cells than B-lymphocytes and macrophages. This pattern of antigen capture in the peripheral non-lymphoid tissues and migration into the draining lymph nodes induces activation and clonal expansion of rare antigen-specific T cells.

Migration of activated DC to the draining lymph nodes has been studied using two experimental approaches to tag migrating DC - (a) skin painting with contact sensitizer fluorescein isothiocyanate (FITC) and tracking FITC positive DC in draining LNs (Macatonia et al., 1987) and (b) genetic immunization with plasmid DNA encoding a reporter such as bacterial β-galactosidase on the abdominal epidermis and tracking

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tagged DC in the draining lymph nodes (Porgador et al., 1998). Both of these approaches have shown that the frequency of antigen-bearing DC that migrate from the peripheral tissues to the draining LN is quite small. Germain and colleagues (Porgador et al., 1998) studied DC migration by gene gun immunization with a cytomegalovirus enhancer/promoter driven β-galactosidase. They showed that majority of antigen-bearing DC were directly transfected with the plasmid DNA and by counting the number of the migrating β-galactosidase expressing DC in the draining LN they concluded that there are approximately 50-100 antigen-bearing DC per LN (Porgador et al., 1998). A major disadvantage with tracking DC in this manner is that the ability to mark DC is dependent upon the promoter activity of β-gal encoding plasmid. Once the promoter activity of the plasmid DNA is extinguished, the DNA plasmid-bearing DC cannot be visualized. We have attempted to overcome this hurdle by permanently marking DC using cre recombinase mediated irreversible genetic recombination.

2. CRE LOX RECOMBINATION TO MARK DC

ROSA26 is a ubiquitous transcriptionally active locus in the mouse genome, which was originally identified by Dr. Soriano and colleagues by using a retroviral gene trapping strategy (Zambrowicz et al., 1997). The ROSA promoter drives very high levels of gene expression in all the tissues tested, embryonic as well as adult (Zambrowicz et al., 1997). Two independent groups have generated novel transgenic mouse strains to monitor Cre-mediated DNA recombination by modifying the ROSA locus (Mao et al., 1999; Soriano, 1999). Cre recombinase is an enzyme made by P1 bacteriophage and it binds to its recognition sequence called loxP. Upon binding to tandem loxP sites, the Cre recombinase catalyzes a genetic recombination event between the loxP sites. Soriano and Mao et al. modified the ROSA locus by introducing a loxP-flanked or neomycin phospho transferase cDNA cassette and the β-gal reporter gene downstream of the ROSA promoter to generate ROSA26R mice (shown in Fig.1). Although the ROSA promoter is transcriptionally active in all cell types and at all developmental stages, the downstream dormant β-gal is not expressed because of the intervening loxP flanked neomycin cassette. β-gal can be expressed only after Cre recombinase mediated excisional recombination of the intervening loxP flanked neomycin cassette (Fig 1) and juxta positioning of the β-gal immediately downstream of the ROSA promoter. Once recombined, β-gal is constantly expressed for the life of the cell (Mao et al., 1999; Soriano, 1999).

To mark DC in ROSA26R mice, we constructed a CMV promoter driven Cre recombinase encoding DNA vaccine (CMV-Cre). To target the recombinase to the nucleus we engineered into the Cre recombinase the nuclear localization signal sequence from simian virus 40 large T antigen. The plasmid DNA was coated on gold bullets and used for immunization. Cohorts of ROSA26R mice were immunized on the abdominal epidermis with a single gene gun immunization of 2μg CMV-Cre and the draining inguinal LNs were analyzed for β-gal expressing antigen presenting cells. At various time points following immunization, expression of β-gal was detected in the draining lymph nodes using the histochemical substrate X-gal (Porgador et al., 1998). β-gal+ cells were observed in the inguinal LNs of mice immunized with CMV-Cre plasmid but not control CMV-influenza hemagglutinin plasmid (Fig.2).