

DENDRITIC CELL MIGRATION AS A REGULATORY FACTOR OF THE IMMUNE RESPONSE

Lymphocytes do not act in isolation, but instead rely upon accessory, antigen-presenting cells (APCs) for initiation and perpetuation of the inflammatory lesion. The most common APCs are the B cell and the Dendritic Cell (DC). While these two cell types differ in many respects, it is generally accepted that the importance of antigen presentation to the immune response is a local event, and therefore almost nothing is known regarding the migratory properties of antigen-sensitized APCs. Current models of dendritic cell (DC) function are based almost exclusively on the Langerhans cell (LC), and predict that once tissue-resident DCs acquire antigen, they alter their functional and surface phenotype (mature) and migrate via the afferent lymphatics to the regional lymph node (MacPherson and Liu, 1999). A small number of B cells recirculate through non-lymphoid tissue, but most B cell recirculation takes place through the lymph node, where antigen-uptake likely takes place. While a large amount of data supports this hypothesis, an apparent paradox exists in the perpetuation of the local response. While DCs are thought to end their life history in the lymph nodes, lymphocytes migrate back to the site of inflammation to clear the antigenic insult. The nature of the antigen presenting cell remaining in the peripheral site remains undefined. Although B cells may leave the lymph node in the efferent lymph, DCs are believed to die *in situ* and never return to the peripheral circulation. No data is currently available regarding the potential role of APC migration in the dissemination or propagation of local immunity. Based on recent evidence, as well as preliminary data from our own laboratory, our hypothesis is that APC migration is a major contributing factor in the initiation and propagation of inflammation. One novel aspect of our hypothesis is that it predicts a subset of antigen-sensitized APCs actively migrates *out* of lymph nodes during inflammation, and eventually (like lymphocytes) migrate back to the original inflammatory site, where they stimulate memory/effector T cells to proliferate and eliminate the infectious insult.

B. Specific Aims

It is the aim of this proposal to define the lymph-borne APCs, both functionally and phenotypically, and to define the migratory mechanisms of afferent and efferent lymph APCs *in vivo*. If our hypothesis is correct, antigen-sensitized APCs will be found in increased numbers in the efferent lymph of lymph nodes draining lesions stimulated with mycobacterial PPD. These APCs should induce an immune response when injected into a distant lymph node, and migrate preferentially back to lymph nodes and inflammatory sites when labeled *in vitro* and reinjected intravenously. Characterization of surface molecules and production of novel antibodies to identify lymph APCs will result in a clear model for the molecular mechanisms involved in these preferential migration patterns. A long term goal of the project is to directly investigate the role of these migrating APCs in the proliferation of inflammation, and eventually the resolution of existing inflammation through the inhibition of mature APC migration back to the inflamed tissue.

In order to test these hypotheses, we have 3 specific aims:

1. *To isolate antigen-sensitized APCs from the afferent and efferent lymph of lymph nodes draining inflammatory sites, and define the kinetics of their appearance in the lymph.*
2. *To define the function and surface phenotype of lymph-borne APCs using existing reagents to investigate the potential mechanism of migratory APCs in inflammation, and to produce novel molecular reagents against this unexplored cell population for later studies.*
3. *To define the migratory potential of afferent and efferent lymph APCs using novel in vivo cell tracking methods, and thereby investigate the mechanism of APC involvement in the perpetuation of inflammation*