Columbia University Opportunity Pool Project: Elucidating the origin and development of bladder resident macrophages

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Introduction

Macrophages are part of the phagocyte mononuclear system constantly replaced by the circulating blood monocytes. In the steady-state, the myeloid cell compartment is highly heterogenous, and contains cells of different origins and functions. These cells include macrophages and dendritic cells, each play important roles in tissue maintenance, including development, homeostasis, immunity and repair following tissue injury.

There have been pivotal studies that have proposed and demonstrated that tissue macrophages originate in part from mesodermal erythro-myeloid progenitors from the yolk sac and has been suggested that the embryonic derived tissue macrophages represent a founding cell type within organ anlagen. These resident macrophages are conserved independently from conventional hematopoiesis by self-renewal and/or longevity.

Macrophages reside in the bladder and are recruited during urinary tract infections. The bladders primary function is to store urine before voiding and to protect the host from toxins that accumulate in the urine, inhibit the invasion of micro-organisms and removal of cellular debris. The multi-functions of the bladder will require this organ to be immunologically unique. **Presently, there are no data on the origins of bladder macrophages, nor a complete understanding of bladder macrophages in the steady state.** Understanding tissue specific immunity in the steady state is essential to fully understand immune responses to immunological insults such as infection, in the case of the bladder urinary tract infections.

Aims

Immunological characterization of the macrophage compartment in murine bladder. Using flow cytometry, we will identify tissue resident and recruited, macrophage and monocyte populations in wildtype male and female mice at 4 time points from prenatal to sexual maturity in the steady state.

Hypothesis: Immunological markers will identify the distinct macrophage populations in the bladder and demonstrate how they vary by age and sex.

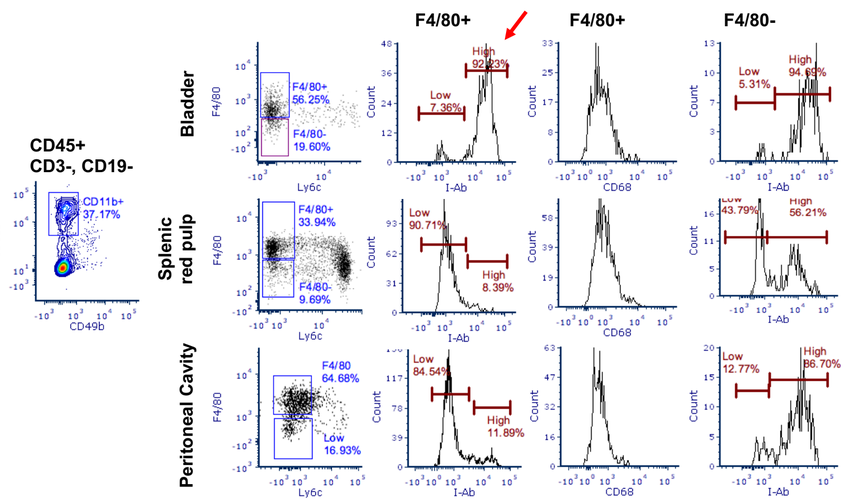
Methods

Bladders were dissociated into a single cell suspension

Cells were stained and analyzed using flow cytometry

Results

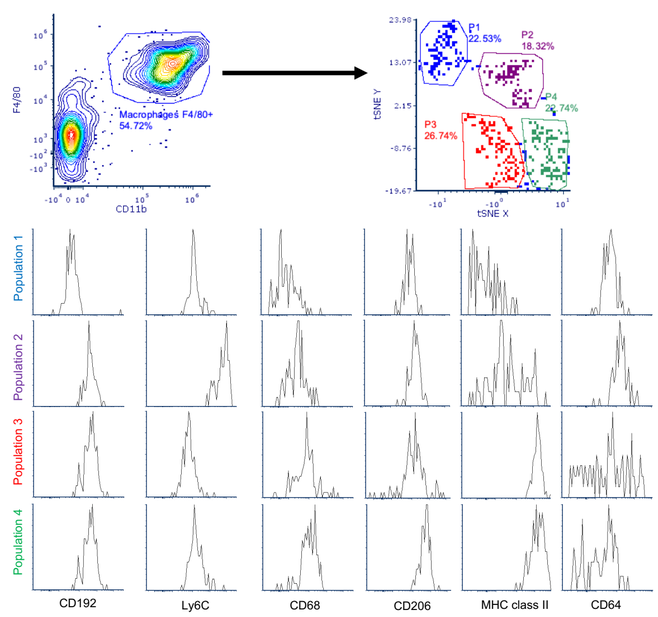
Phenotypic analysis of bladder (top), splenic red pulp (middle) and peritoneal cavity macrophages (bottom) in adult female C57BL/6 mice



CD45+, CD3-, CD19-, CD49b-, CD11b+ cells were initially examined for F4/80 surface staining. The expression of I-A^b was determined on the F4/80+ and F4/80-. CD68 expression was determined on the F4/80+ macrophage populations. Arrow denotes unique bladder macrophage population.

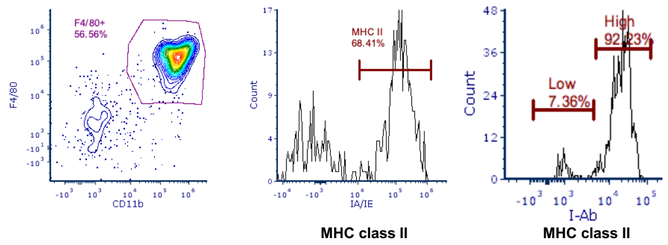
F4/80 - tissue macrophage marker, Ly6C - monocyte marker, CD68 - tissue macrophage marker, I-A^b - MHC class II

Four distinct macrophage populations in the bladders of 3-week-old mice



There are four distinct resident macrophage populations identified by flow cytometry including resident and recruited macrophage populations. The four distinct macrophage populations may have unique functional properties.

Bladder macrophages shift to either an activated or recruited population in adult female mice



In Summary

We will have an in-depth immunological characterization of the macrophage compartment in murine bladder, prenatally through sexual maturity in C57BL/6 male and female mice

Macrophage populations in the bladder are heterogenous

In mice at three weeks of age we are able to distinguish both resident and recruited macrophages.

In female adult mice the bladder macrophages are nearly all MHC class II high indicating:

- **all macrophages are activated**
- **the resident macrophage population is replenished by the recruited monocyte derived macrophages**

Future Work

The transcriptomic analysis: This will identify specific genes and transcription factors unique to the bladder macrophage populations that will help elucidate their function and origin. In addition, we will be able to identify preferentially expressed transcription factors which may be key in bladder macrophage development. This data will allow us to develop hypothesis on the lineage of bladder resident macrophage populations to develop *in vivo* fate mapping studies

Funding

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