Targeting critical immune cell populations in pulmonary metastasis using novel monocyte-focused therapy

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BACKGROUND

Cancer research in the Headley Lab focuses on the immune system, as it pertains to the formation of metastasis and tumor survival. The immune system plays a dual role in tumor pathology with some elements recognizing and eliminating cancer while others support tumor growth. Pulmonary metastasis is a lethal development for many cancer types, made possible in part by pre-tumor monocyte populations within the pre-metastatic niche. I am investigating therapeutic targeting of conventional monocytes in pulmonary metastatic cancer prevention. Conventional monocytes can differentiate into distinct populations with either anti or pro-tumor function.

Figure 1: Role of The Immune System in Tumor Metastasis

We have recently developed a novel Monoclonal antibody (Mono1) that depletes conventional monocytes but not other cell populations. This reagent allows us to interrogate the function of conventional monocytes in vivo in ways that were previously impossible. In order to effectively this tool it is critical that we first understand the mechanisms by which it depletes conventional monocytes so we can properly interpret results in future experiments. Known pathways for immune cell depletion include antibody dependent cellular cytotoxicity (ADCC) and antibody dependent cellular phagocytosis (ADCP). In the studies presented here we explored mechanism behind Mono1-mediated depletion of conventional monocytes. Intriguingly, despite robust depletion of only conventional monocytes we show that Mono1 does not in fact bind to conventional monocytes directly. Based on this unexpected result we next investigated the role of the other functional element on antibodies, the Fc region in Mono1-mediated depletion of conventional monocytes. Canonical ADCC and ADCP require recognition of the Fc region of the depleting antibody by activating Fc Receptors on effector cells, thus we have explored the requirement for Fc receptor signaling in mediating the monocyte-depleting effect of Mono1 in vivo. My work has played a crucial role in the advancement of preventative immunotherapy and provided useful data moving forward with Mono1 research, though more studies are required to define the mode of action of this intriguing new tool.

Figure 2: Proposed Model of Conventional Monocyte Depletion

METHODS

Antibody Injections

W7, FcRgKO, and FcRgKo mice (as indicated for given experiments) were injected intraperitoneally with 15mg/kg of Mono1 or Isotype Control (mIgG2a) antibody at time zero

Long Term Harvesting and Flow Cytometry Protocol

1. At 24 hr mice were injected intraperitoneally with 1ml of 10% Avertin (in PBS).
2. Lung tissue was collected from each mouse and digested using a DNAaseliberase enzyme solution and GentleMACS’s blender tube to create a single cell suspension.
3. Approximately 5 million cells were resuspended with FACS buffer in a 96 well plate.
4. Cells were stained with eFlour780 Live/Dead antibody then treated with FcBlock solution to prevent non-specific binding to the FcRg activating receptors.
5. Cells stained with 22 fluorescent antibody markers and 5ml of countinventive heads for flow cytometry on the BD FACSymphony.
6. Critical immune cell populations were analyzed for all 31 mice lung samples using 13 designated laser excitation channels.

RESULTS

Figure 4: In-Vivo Mono1 Antibody Specifically Depletes Conventional Monocytes

Figure 5: Mono1 Selectively Binds to Neutrophils but not Conventional Monocytes ex vivo and in vivo

Figure 6: Mono1-depletion is FcRg-dependent

CONCLUSIONS

- Mono1 specifically depletes conventional monocytes in vivo
- Mono1 does not bind conventional monocytes in vitro
- Mono1 robustly labels neutrophils in vivo and ex vivo
- Mono1-mediated depletion of conventional monocytes is activating Fc Receptor dependent

FUTURE DIRECTIONS

- What is the effector cell population required for Mono1-mediated depletion of conventional Monocytes?
  - Do Neutrophils mediate conventional monocyte depletion via an indirect mechanism
  - Alternatively, is another effector cell population required (e.g. NK cells) for Mono1-mediated depletion of conventional monocytes
- Ascertain long term effect of monocyte depletion to track turnover rate in downstream populations
- Interrogate the role of conventional monocytes specifically in diseases such as metastasis of breast cancer to lungs.

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