Interrogating the Localisation and Maturational State of Neutrophils in the Lymph Node

Student: Alexander Young
Supervisor: Dr Leo Carlin
Cancer Research UK Beatson Institute

Background

Neutrophils are key effector cells in the immune system. Normally, they are important first responders against infection and are predominantly found in blood and peripheral tissues, but they can also be found within lymph nodes (LNs). LNs are vital tissues for providing an environment to facilitate the coordination of immune responses to diseases such as cancer. In the context of cancer tumour-draining LNs (tdLNs) play a vital role in the anti-tumour immune response. Neutrophils have been implicated in having pro-tumoural effects due to their role in promoting angiogenesis, metastasis and the suppression of adaptive immune responses.
It is unclear what the role of neutrophils are within the tdLN. Understanding their maturational state, tissue localisation and cell interactions in LNs is fundamental to understanding their function.

Aims

To visualise neutrophils within lymph nodes and determine their maturational state and activation status within this environment.

Methods

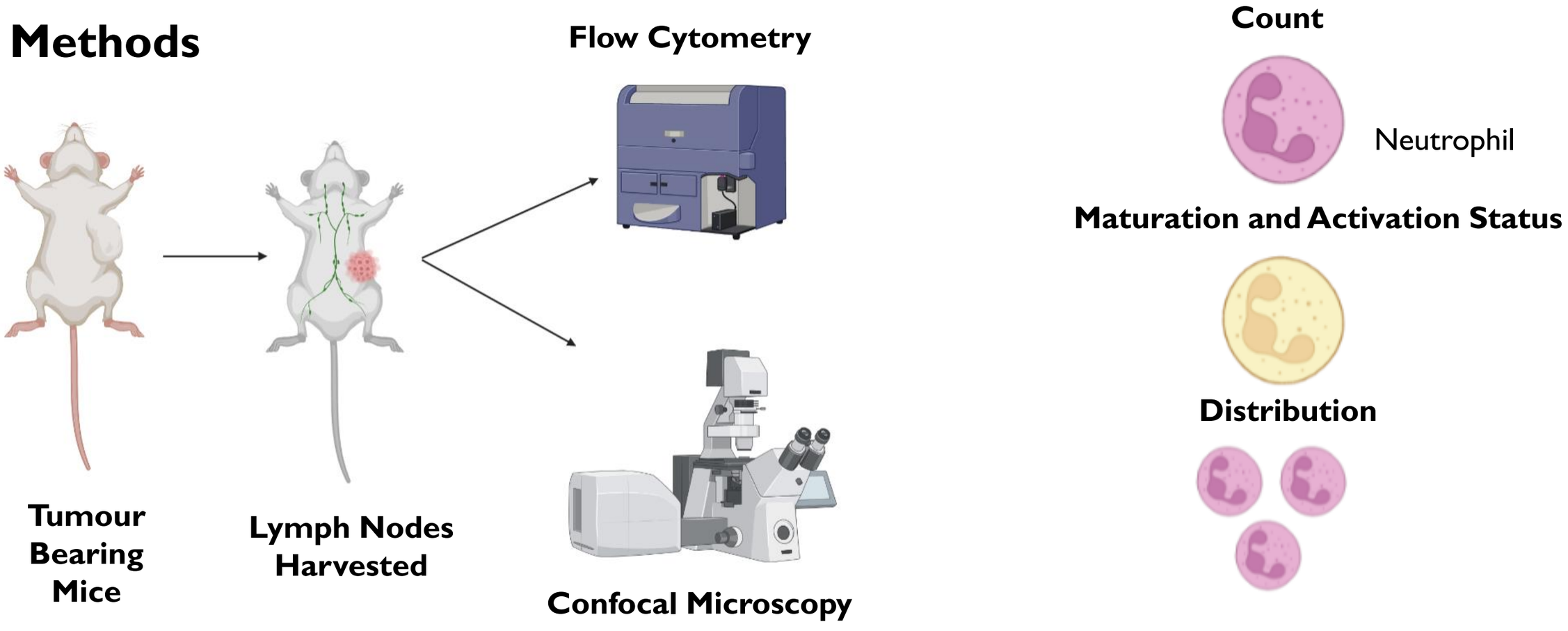


Figure 1: *Summary of methods.* *Axillary, inguinal and nediastinal LNs were harvested from WT and tumour bearing mice. LNs were processed for flow cytometry and confocal microscopy. Count, maturation status and activation state were quantified using flow cytometry and validated with confocal microscopy. Confocal microscopy was also used to visualise neutrophils within lymph nodes. Neutrophil maturity can be determined using Ly6G expression and their activation status was determined using CD11B expression.*

Results

Decrease in Neutrophils in the Mediastinal Lymph Node in Tumour Bearing Mice

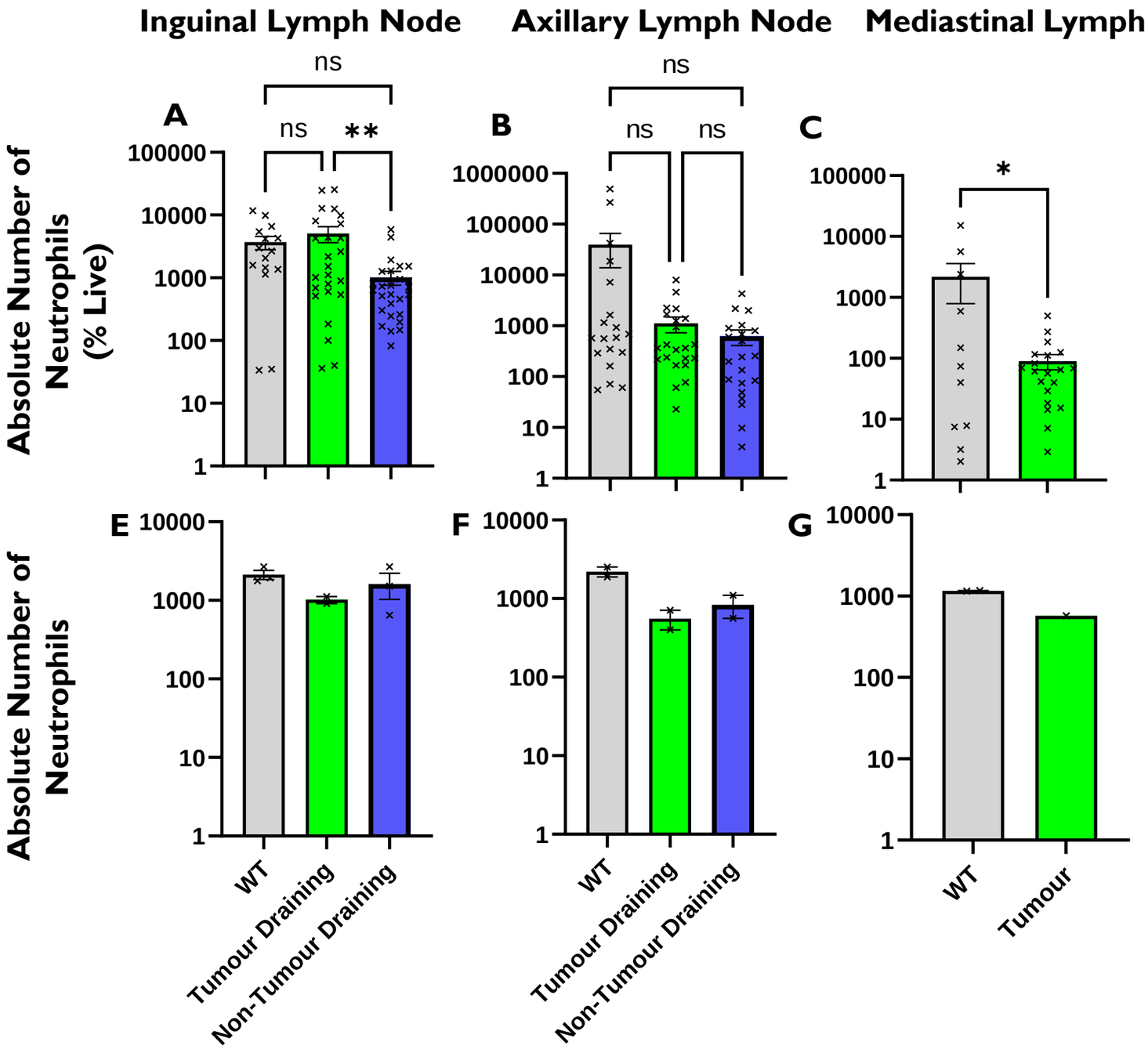


Figure 2: ***Absolute Number of Neutrophils Within Lymph Nodes. A-C)*** *The Neutrophil compartment from WT, tumour draining and non-tumour lymph nodes was analysed using flow cytometry (Gating strategy in supplementary) and the absolute number of neutrophils were quantified. No significant difference in neutrophil numbers was observed. Data represent absolute number ± SEM from 7 independent experiments for flow cytometry.* ***D)*** *Representative image of WT Axillary LN.* ***E-G*** *Quantification of neutrophils from confocal imaging. For A and B (one way ANOVA with multiple comparisons). For C p<0.05*,<0.01** (un-paired t test)*

Significant decrease in mature neutrophils in the mediastinal lymph node of tumour bearing mice

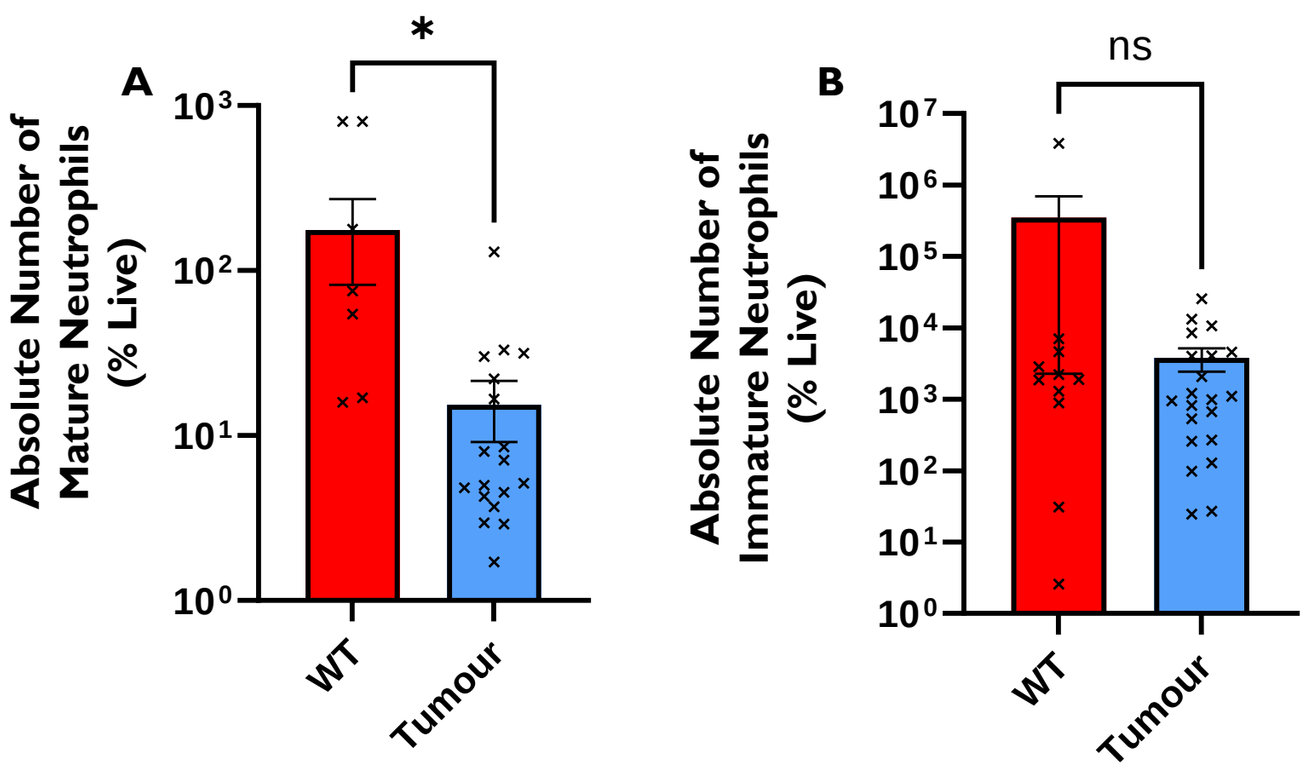


Figure 3: ***Within the mediastinal lymph node in tumour bearing mice there is a decrease in the number of mature neutrophils.*** *The maturation state of neutrophils within WT and tumour draining lymph nodes was determined. Mature neutrophils were defined as (Ly6GHI) and immature as (Ly6GLO). There was no significant difference in maturation state of neutrophils within auxiliary and inguinal lymph nodes (data not shown). For A and B p<0.05 * (un-paired t test).*

No significant difference in the activation state of mature and immature neutrophils in mediastinal lymph node

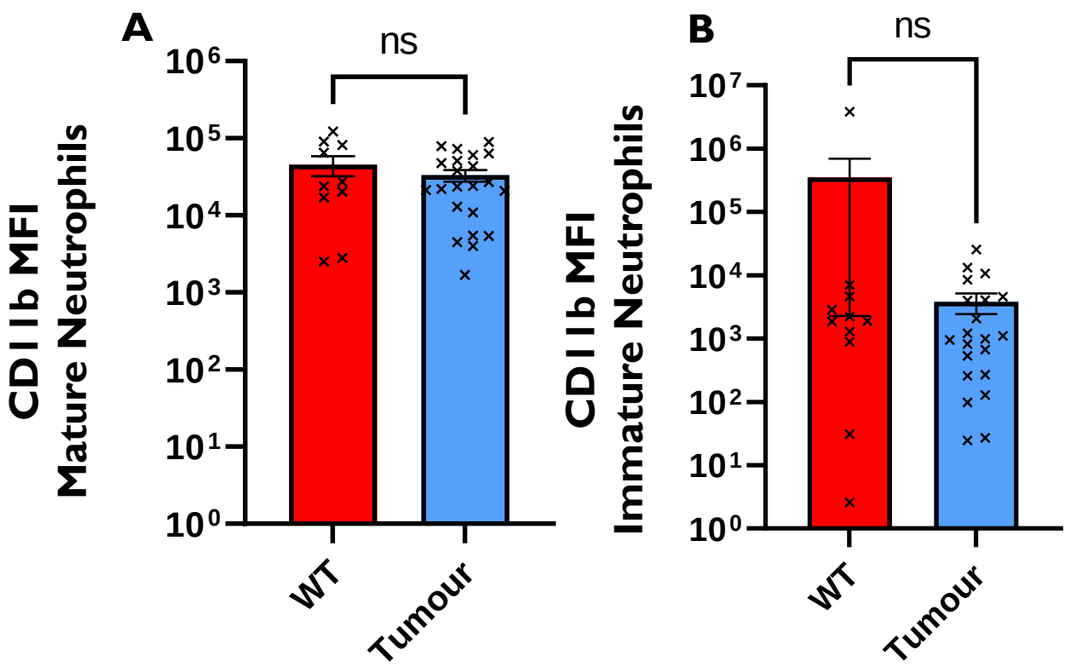


Figure 4: ***Within the mediastinal lymph node in tumour bearing mice there is no difference in neutrophil activation status.*** *The activation status of mature (Ly6GHI) and immature neutrophils (Ly6GLO) within the mediastinal lymph node was determined using CD11b expression. There was no significant difference CD11b expression.There was no difference in axillary and inguinal lymph nodes(data not shown). For A and B (un-paired t test)*

Clustering of Tumour Antigen Bearing Neutrophils within the Lymph Node

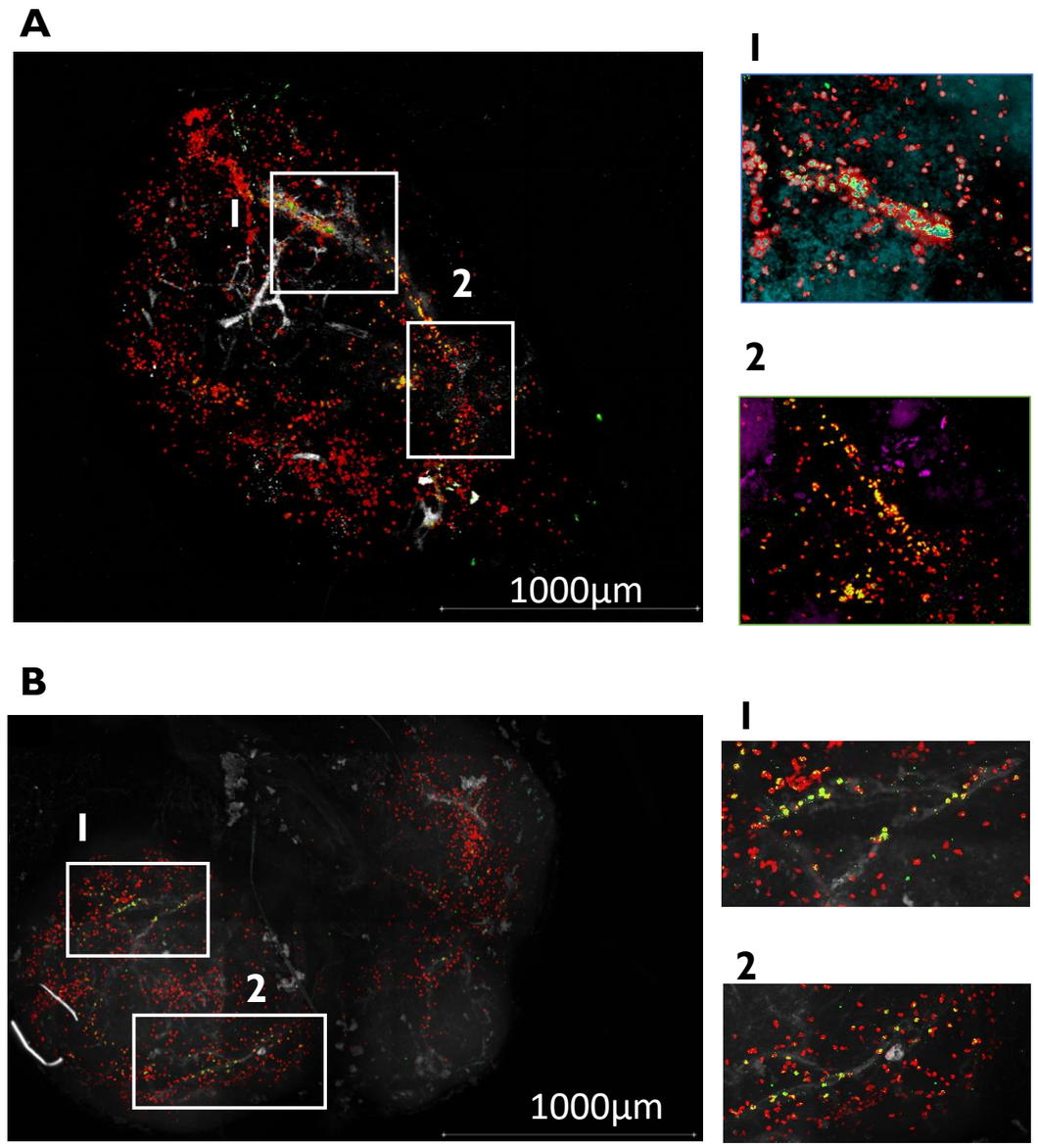


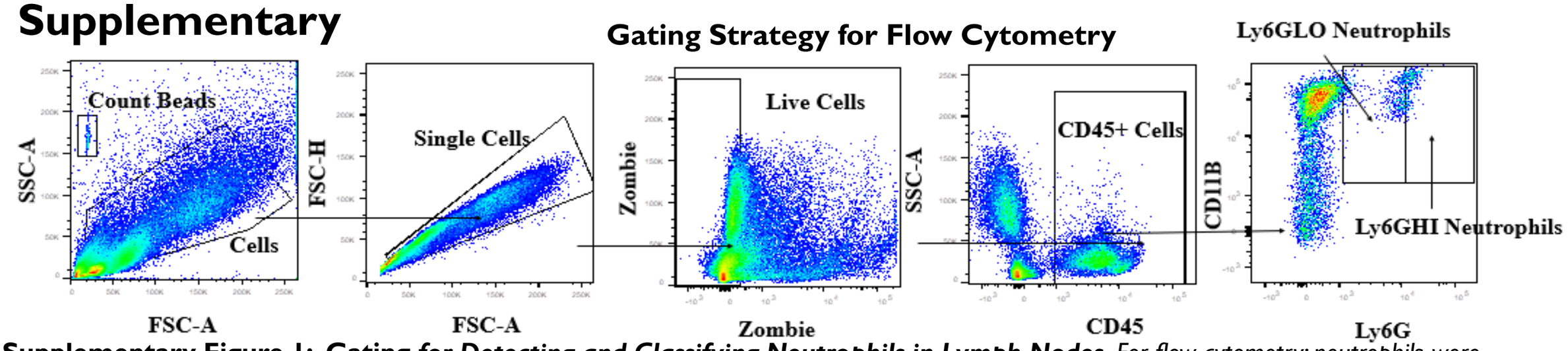
Figure 5: ***Clustering of tumour antigen loaded neutrophils within the tumour-draining axillary lymph node and non-draining inguinal lymph node. A)*** *Tumour draining axillary lymph node and* ***B)*** *non draining inguinal lymph nodes were imaged with confocal microscopy for neutrophils (red), tumour Antigen (green),T Cells (Pink), B Cells (Blue). Lymph node structure and vasculature is shown in grey colours. Sections of interest were enlarged to better visualise areas of clustered neutrophils.*

Conclusions

These results suggests that tumour bearing mice have alterations in their neutrophil populations within their tdLN in terms of in their maturational status and activation state. There is currently limited understanding as to the functional significance of neutrophil maturation within lymph nodes. It will be interesting to understand how these altered neutrophils populations impact the generation of adaptive immunity, particularly T cells responses, within the tdLN. Further studies should seek to clarify phenotypic markers of neutrophil maturation and to determine their cellular interacting partners.

Supplementary

Gating Strategy for Flow Cytometry



Supplementary Figure 1: Gating ***for Detecting and Classifying Neutrophils in Lymph Nodes.*** *For flow cytometry: neutrophils were gated as (Zombie-,CD45+, CD11B+, Ly6G+), Immature Neutrophils were gated as (Zombie-,CD45+, CD11B+, Ly6GLo) and Mature Neutrophils were gated as (Zombie-, CD45+, CD11B, Ly6GHI).*

Acknowledgements

Zsombor Kerekes, Chiara Prillo, Carlin Lab Roberts Lab (R24), Tom Gilbey, Yi-Hsia Liu, Central Services, BSU/BRU