



University
of Manitoba



Detecting LAG3: antibody discrepancies in flow cytometry

Colin G Graydon¹, Monika Kowatsch¹, Allison Balasko¹, Julie Lajoie^{1,3}, Keith R Fowke^{1,2,3,4}

¹Department of Medical Microbiology and Infectious Diseases, University of Manitoba

²Department of Community Health Sciences, University of Manitoba

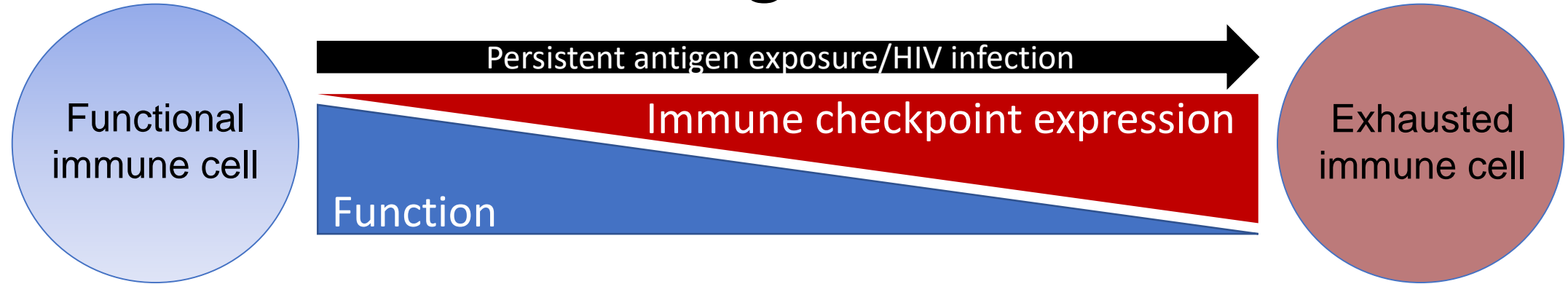
³Department of Medical Microbiology, University of Nairobi

⁴Partners for Health and Development in Africa

Questions? Comments? Contact Colin at graydonc@myumanitoba.ca

Conflict of Interest Disclosure: I have no conflicts of interest

Background



LAG3 is an immune checkpoint that...

... is upregulated during HIV¹

... may enhance HIV latency by inhibiting activation and killing of infected cells²⁻⁴

... is a potential target for HIV functional cure⁵

However, LAG3 expression is...

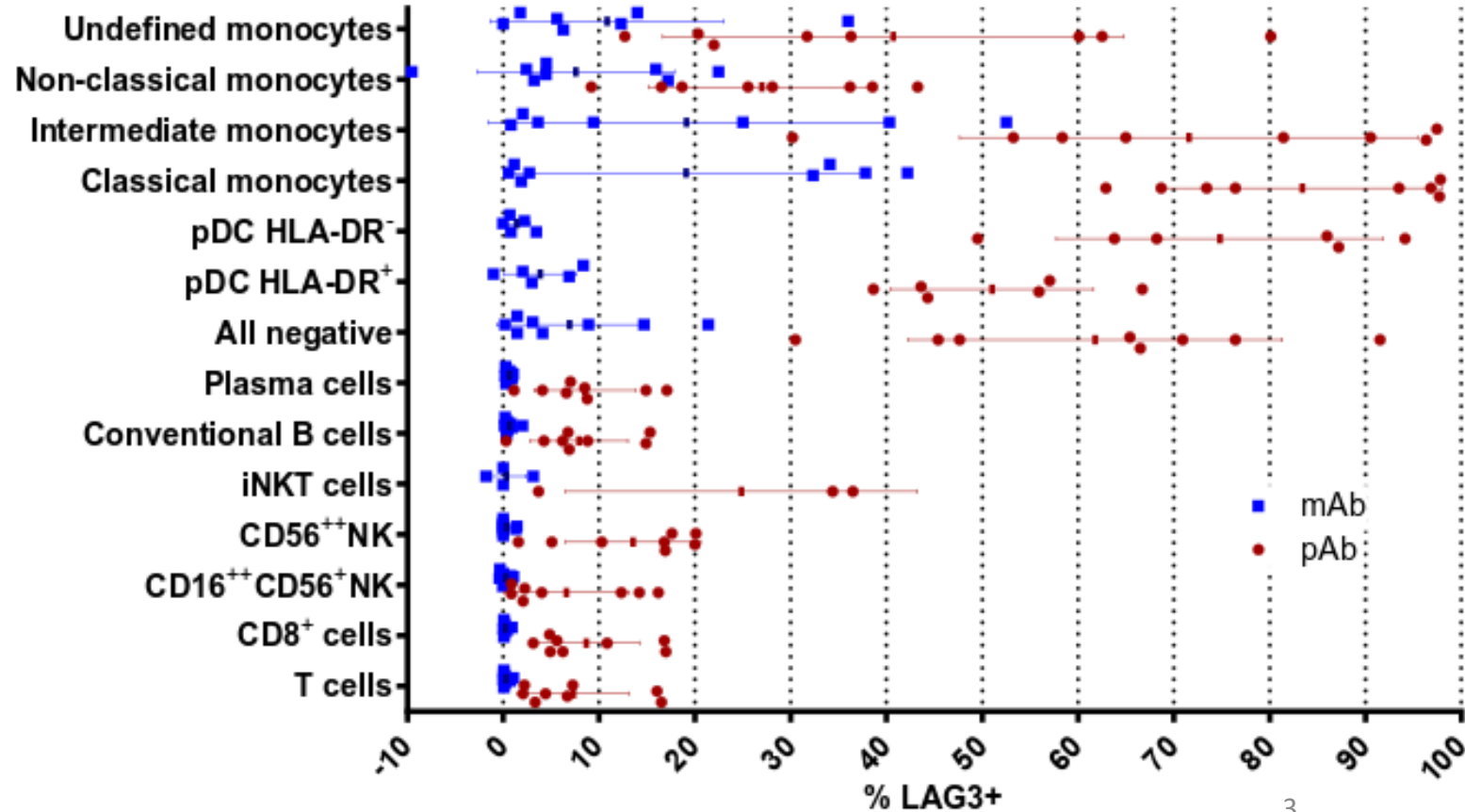
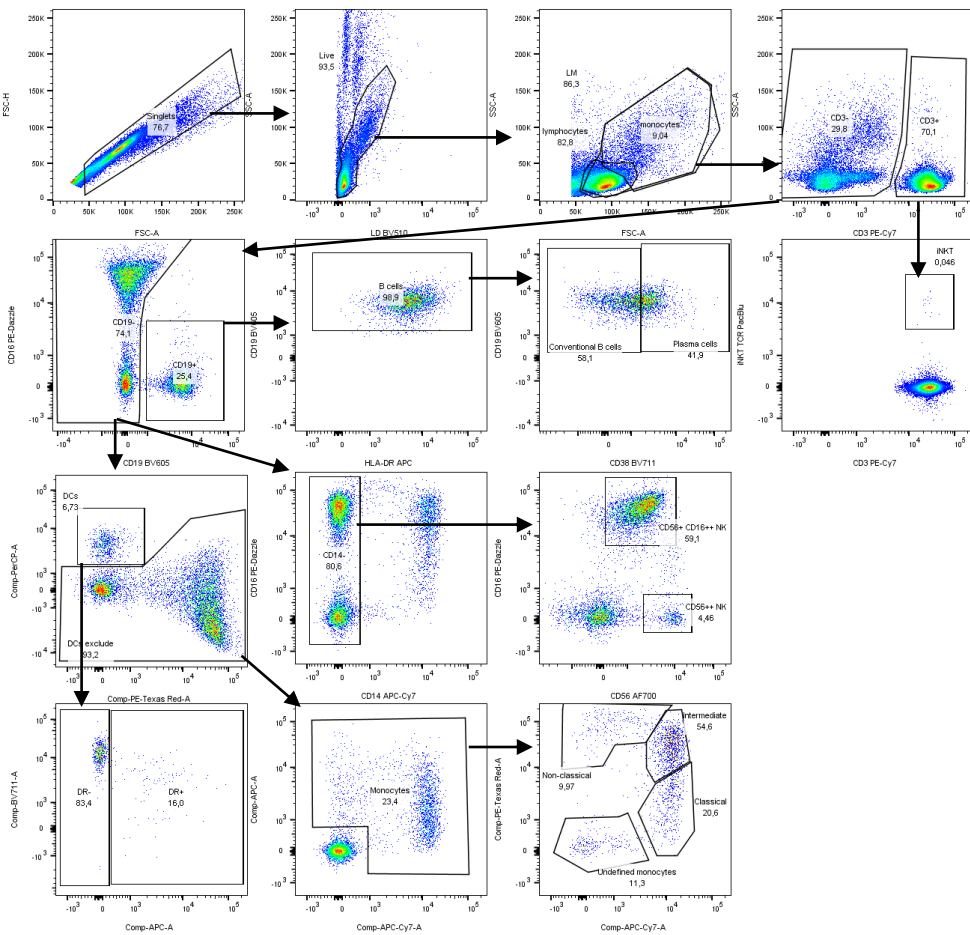
... unreported on many cell types

... highly variable in the literature, which coincides with use of either monoclonal or polyclonal antibodies (mAbs or pAbs)

Anti-LAG3 pAb binds at higher levels than mAb AND monocytes may express high levels of LAG3

Methods:

Healthy donor PBMC were stained with LAG3 mAb and pAb in addition to phenotype markers. In a separate experiment 3 separate lots of pAb similar results (data not shown)



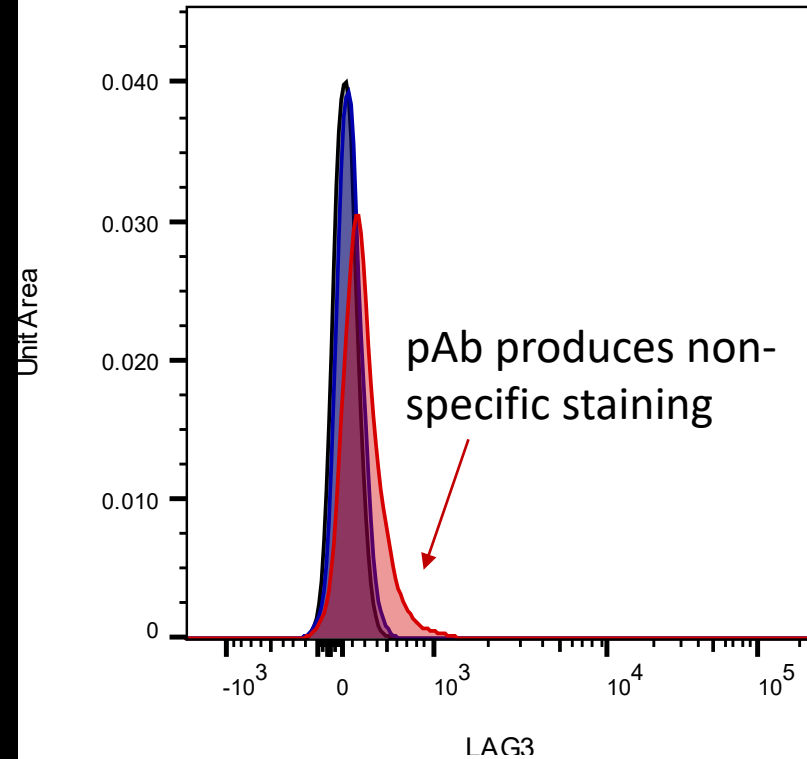
pAb exhibits greater non-specific binding than mAb

Methods:

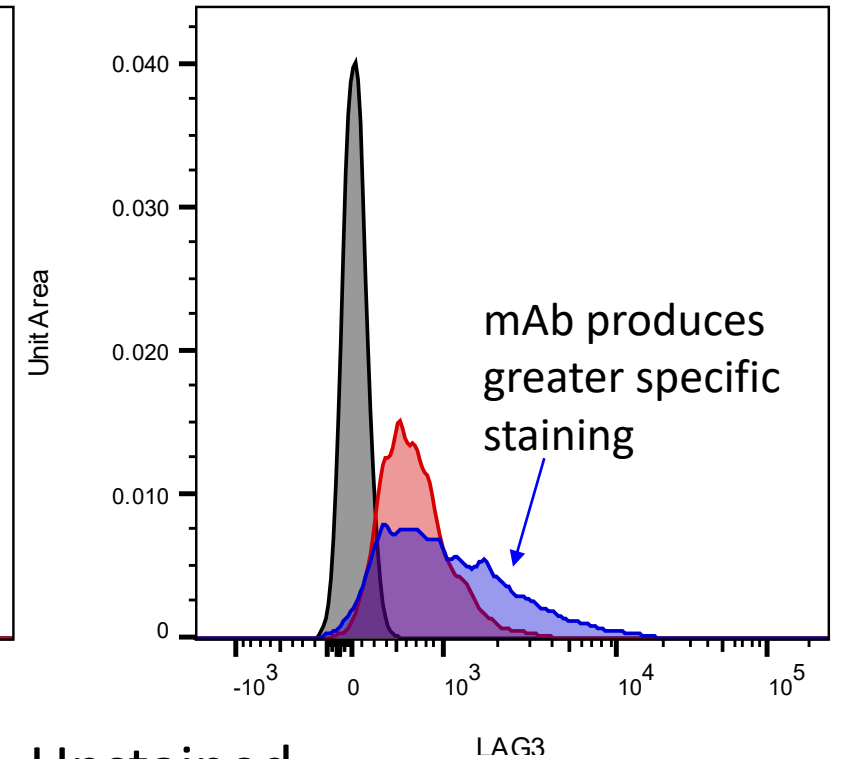
Jurkat T cell line was transduced with lentiviral vector carrying a plasmid containing a LAG3 insert (LAG3⁺), or a control plasmid lacking LAG3 (LAG3⁻).

These cells were stained with either an anti-LAG3 mouse IgG monoclonal antibody (BD, clone: T47-530), or the anti-LAG3 goat polyclonal antibody (R&D, Cat #: FAB2319P).

LAG3⁺ Jurkats



LAG3⁻ Jurkats



- Unstained
- mAb
- pAb

Conclusions

- Commonly used anti-LAG3 antibodies have very different binding profiles.
 - The commonly used anti-LAG3 goat polyclonal antibody binds to PBMC at higher levels than monoclonal antibodies, potentially as a result of non-specific binding.
- Monocytes may express high levels of LAG3

References

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Significance

- In interpreting LAG3 expression levels from the literature, LAG3 antibody clone should be noted.
- Comparisons between studies using polyclonal and monoclonal anti-LAG3 may not be valid.
- LAG3 expression on monocytes may represent a novel regulatory mechanism for these cells.
 - This has implications for:
 - ❖ Reversal of HIV latency in monocytes
 - ❖ Potential novel cell type specific LAG3 functions
 - ❖ Greater appreciation for breadth of impact of checkpoint blockade

