Cattle T Cell Phenotyping by an 8-Colour, 10-Parameter Panel

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Abstract:

This multiplex staining panel was developed to differentiate cattle T cells into conventional (CD4 and CD8) and unconventional (γδ-TCR) subsets as well as their stage of differentiation and activation. The combination of CD45RO and CD62L allows the identification of naïve ($T_{Naïve}$), central memory (T_{CM}), effector memory (T_{EM}) and terminal effector (T_{TE}) T cells. Activated cattle T cells (T_{AV}) can be identified by the cell surface expression of CD25. This panel was developed using cryopreserved cattle peripheral blood mononuclear cells (PBMCs) and tested on fresh as well as stimulated PBMCs. Therefore, this 8-colour, 10-parameter flow cytometry panel simultaneously identifies cattle $T_{Naïve}$, T_{AV}, T_{CM}, T_{EM}, T_{TE} and γδ-TCR cells. This panel will improve our ability to examine T cell response to pathogens and vaccines in cattle including the potential to identify previously undescribed subpopulations. Furthermore, this panel can be readily optimised for other bovid species as many of these reagents are likely to cross react.

Key terms:

Flow cytometry; cattle PBMC; T cells; naïve T cells; effector memory T cells; central memory T cells; activated T cells; γδ T cells; T cell subsets

Background:

Robust T cell responses are critical in the response to pathogen infection both for clearance and the formation of strong and broad memory responses (1). Cattle, like several other species, have a much higher proportion of γδ T cells compared to CD4 and CD8 (2–4). Consequently, it is important to study the entire T cell compartment simultaneously to fully characterise how immune protection arises and persists. Furthermore, as the research climate focusses on One Health approaches, the ability to study the immune response at high resolution in species that underpin global food security is essential.

Common to several non-model species, the first mAbs to study CD molecules on cattle T cells were derived from mouse immunizations with whole cattle PBMC populations or PBMC lysates. Antibodies were characterized in three international workshops on ruminant antigens (2,5,6). Together with identification of cross-reactive mAbs, this allowed the establishment of a basic toolbox to study cattle T cells and various subsets within them (7). However, several limitations still exist for the establishment of polychromatic flow cytometry staining panels. For example, many of the current 371 human CD molecules do not have an antibody that cross react with cattle. Another major limitation is the lack of useful mAbs that are labelled to a wider range of fluorochromes. This makes it difficult to expand panels beyond the three most common fluorochromes FITC (or AF488), PE and APC (or AF647). By conjugating existing T cell markers in-house we were able to develop a

cattle T cell panel that utilises eight colours excluding PE and APC-conjugated antibodies. This allows the addition of specific antibodies, such as for cytokines or transcription factors, that maximises the broader utility of this panel for individual research needs. Additionally, if more of the available mAbs would be conjugated to fluorochromes that are excited by the violet laser, the panel can be further expanded.

This OMIP identifies all three main cattle T cell subsets (CD4, CD8 and γδ), as well as their subsets that are activated (T_{AV}) or in the distinct differentiation states of naïve ($T_{Naïve}$), central memory (T_{CM}), effector memory (T_{EM}) and terminal effector (T_{TE}). The gating strategy we used initially identifies the two αβ T cell subsets CD4 (mAb clones CC8 / CC30) and CD8 (mAb clone CC63) as well as the γδ T cells (mAb clone GB21A) (2,8) (Fig. 1). Like in swine and chickens, γδ T cells constitute a major T cell subset in cattle blood and can comprise more than 50% of circulating T cells (3,9). To identify activated T cells, CD25 (mAb clone IL-A111) can be used (8,10–12), whereas the memory state of the cells can be defined using the CD45RO (mAb clone IL-A116) and CD62L (mAb clone CC32) cell surface markers (6,8,13–15) (Fig. 1). Using this gating strategy, the following known subsets can be identified for the helper T cells, $T_{Naïve}$ ($CD3^+γδ\text{-}TCR^-CD4^+CD25^-CD45RO^-CD62L^+$), T_{CM} ($CD3^+γδ\text{-}TCR^-CD4^+CD25^-CD45RO^+CD62L^+$), T_{EM} ($CD3^+γδ\text{-}TCR^-CD4^+CD25^-CD45RO^+CD62L^-$), T_{TE} ($CD3^+γδ\text{-}TCR^-CD4^+CD25^-CD45RO^-CD62L^-$) and T_{AV} ($CD3^+γδ\text{-}TCR^-CD4^+CD25^+$). Similarly, the cytotoxic T cells can be separated into $T_{Naïve}$ ($CD3^+γδ\text{-}TCR^-CD8α^+CD25^-CD45RO^-CD62L^+$), T_{CM} ($CD3^+γδ\text{-}TCR^-CD8α^+CD25^-CD45RO^+CD62L^+$), T_{EM} ($CD3^+γδ\text{-}TCR^-CD8α^+CD25^-CD45RO^+CD62L^-$), T_{TE} ($CD3^+γδ\text{-}TCR^-CD8α^+CD25^-$

CD45RO$^-$CD62L$^-$) and T_{AV} (CD3$^+$γδ-TCR$^-$CD8α$^+$CD25$^+$). Furthermore, γδ T cells can also be identified by (CD3$^+$γδ-TCR$^+$) (Fig. 1; Online Table 3). A major improvement by this panel is the simultaneous analysis of all these T cell subsets in a single sample, hence reducing the variation between replicates and the number of samples needed per animal.

The panel was designed and optimised on a BD LSRFortessa and was tested on a BD Aria IIIU. The BD Aria IIIU allows for sorting of the T cell subsets. Further adaptations to the panel are enabled by having both the PE and APC channel empty, for which many antibodies are commercially available. If more reagents become available in the violet channel, they can easily be added to the panel with only minor influence on compensation requirements.

In conclusion, this cattle T cell panel will advance the understanding of the cattle immune response as it allows the measurement of all major T cell subsets and their differentiation stage within a single sample.

Similarity to published OMIPs:

None to date.

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103 **Literature Cited**

104 1. Laidlaw BJ, Craft JE, Kaech SM. The multifaceted role of CD4+ T cells in CD8+ T
105 cell memory. Nat. Rev. Immunol. 2016;16:102–111.
106 2. Davis WC, Brown WC, Hamilton MJ, Wyatt CR, Orden JA, Khalid AM, Naessens
107 J. Analysis of monoclonal antibodies specific for the γδ TcR. Vet. Immunol.
108 Immunopathol. 1996;52:275–283.
109 3. Baldwin CL, Damani-Yokota P, Yirsaw A, Loonie K, Teixeira AF, Gillespie A.
110 Special features of γδ T cells in ruminants. Mol. Immunol. 2021;134:161–169.
111 Available at: https://doi.org/10.1016/j.molimm.2021.02.028.
112 4. Telfer JC, Baldwin CL. Bovine gamma delta T cells and the function of gamma
113 delta T cell specific WC1 co-receptors. Cell. Immunol. 2015;296:76–86. Available at:
114 http://dx.doi.org/10.1016/j.cellimm.2015.05.003.
115 5. Howard CJ, Morrison WI, Bensaid A, Davis W, Eskra L, Gerdes J, Hadam M,
116 Hurley D, Leibold W, Letesson J-J, MacHugh N, Naessens J, O'Reilly K, Parsons KR,
117 Schlote D, Sopp P, Splitter G, Wilson R. Summary of workshop findings for
118 leukocyte antigens of cattle. Vet. Immunol. Immunopathol. 1991;27:21–27. Available

119 at: https://linkinghub.elsevier.com/retrieve/pii/016524279190072K.

120 6. Naessens J, Hopkins J. Introduction and summary of workshop findings. Vet.

121 Immunol. Immunopathol. 1996;52:213–235. Available at:

122 https://linkinghub.elsevier.com/retrieve/pii/0165242796055663.

123 7. Sopp P, Werling D, Baldwin C. Cross-reactivity of mAbs to human CD antigens

124 with cells from cattle. Vet. Immunol. Immunopathol. 2007;119:106–114. Available at:

125 https://linkinghub.elsevier.com/retrieve/pii/S0165242707002206.

126 8. Howard CJ, Naessens J. 4.1 Summary of workshop findings for cattle (Tables 1

127 and 2). Vet. Immunol. Immunopathol. 1993;39:25–47. Available at:

128 https://linkinghub.elsevier.com/retrieve/pii/016524279390161V.

129 9. Baldwin CL, Telfer JC. The bovine model for elucidating the role of γδ T cells in

130 controlling infectious diseases of importance to cattle and humans. Mol. Immunol.

131 2015;66:35–47. Available at: http://dx.doi.org/10.1016/j.molimm.2014.10.024.

132 10. Coussens PM, Sipkovsky S, Murphy B, Roussey J, Colvin CJ. Regulatory T cells in

133 cattle and their potential role in bovine paratuberculosis. Comp. Immunol.

134 Microbiol. Infect. Dis. 2012;35:233–239. Available at:

135 http://dx.doi.org/10.1016/j.cimid.2012.01.004.

136 11. Caruso A, Licenziati S, Corulli M, Canaris AD, De Francesco MA, Fiorentini S,

137 Peroni L, Fallacara F, Dima F, Balsari A, Turano A. Flow cytometric analysis of

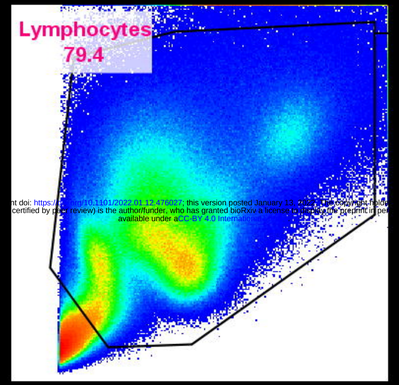
138 activation markers on stimulated T cells and their correlation with cell proliferation.

139 Cytometry 1997;27:71–6. Available at:

140 http://www.ncbi.nlm.nih.gov/pubmed/9000587.

141 12. Waters WR, Rahner TE, Palmer M V., Cheng D, Nonnecke BJ, Whipple DL.

142 Expression of L-selectin (CD62L), CD44, and CD25 on activated bovine T cells.
143 Infect. Immun. 2003;71:317–326.
144 13. Golubovskaya V, Wu L. Different subsets of T cells, memory, effector functions,
145 and CAR-T immunotherapy. Cancers (Basel). 2016;8.
146 14. Bembridge GP, MacHugh ND, McKeever D, Awino E, Sopp P, Collins RA,
147 Gelder KI, Howard CJ. CD45RO expression on bovine T cells: relation to biological
148 function. Immunology 1995;86:537–44. Available at:
149 http://www.ncbi.nlm.nih.gov/pubmed/8567018%0Ahttp://www.pubmedcentral.
150 nih.gov/articlerender.fcgi?artid=PMC1384052.
151 15. Whelan AO, Villarreal-Ramos B, Vordermeier HM, Hogarth PJ. Development of
152 an antibody to bovine IL-2 reveals multifunctional CD4 T EM cells in cattle naturally
153 infected with bovine tuberculosis. PLoS One 2011;6:2–9.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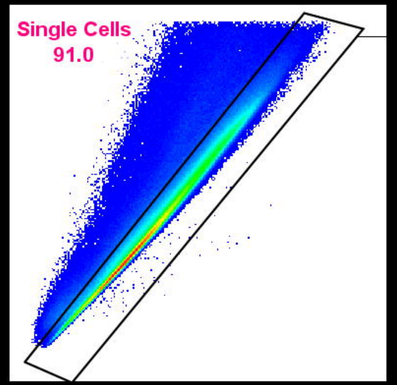
79.4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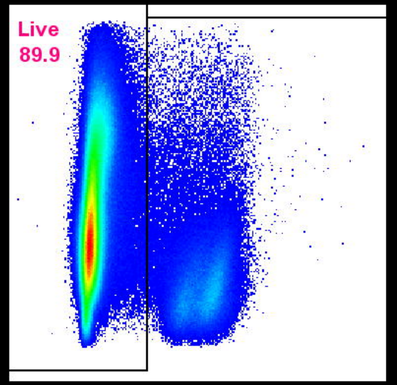
Live
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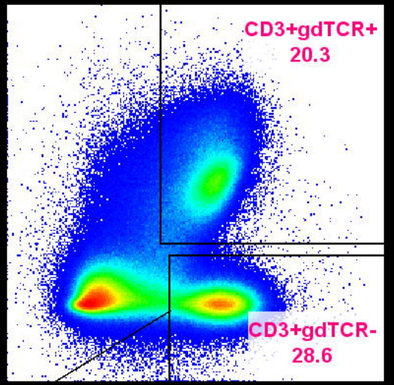
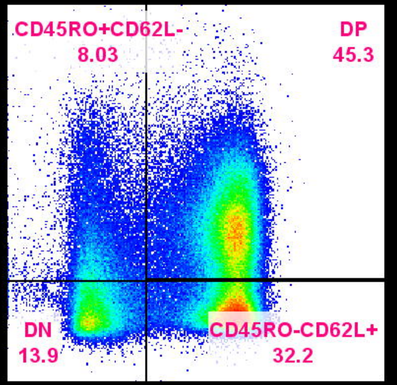
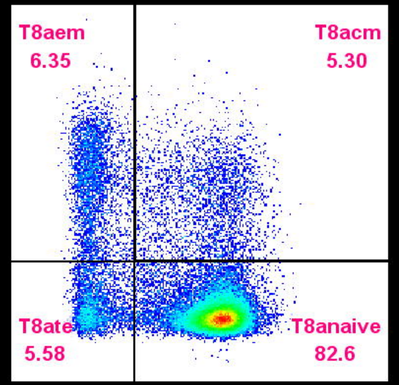

CD3+gdTCR+
20.3
CD3+gdTCR-
28.6


CD45RO+CD62L-
8.03
DP
45.3
DN
13.9
CD45RO-CD62L+
32.2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T8acm
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T8anaive
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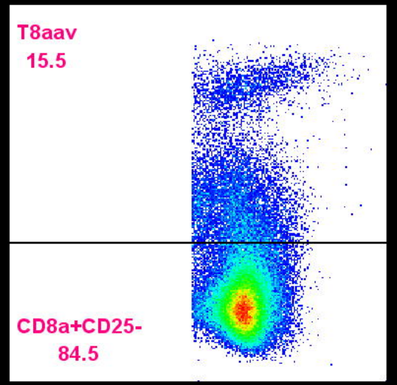
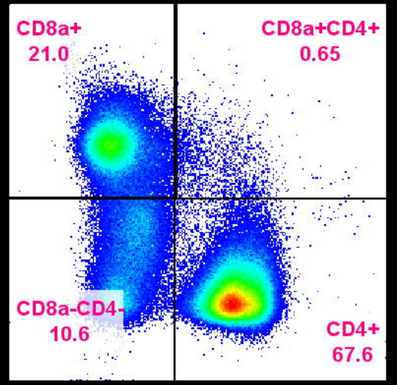

T8aav
15.5
CD8a+CD25-
84.5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CD8a+CD4+
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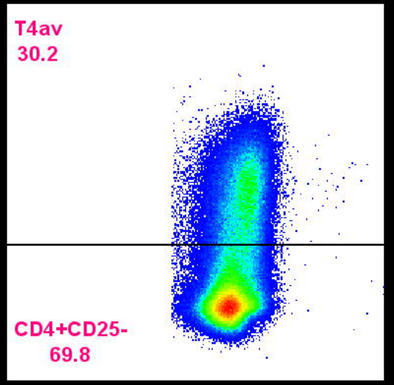
CD8a-CD4-
10.6
CD4+
67.6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CD4+CD25-
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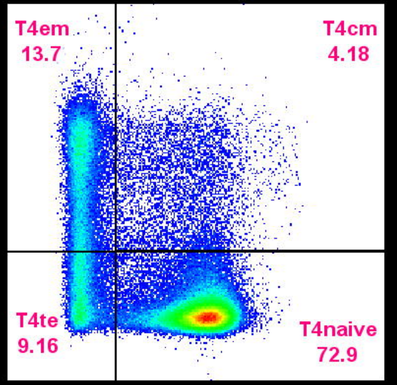
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T4te
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72.9