IGF-1R Expression Correlates with an Inflammatory Phenotype in Canine Monocytes

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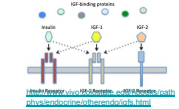
Abstract

Insulin-like Growth Factor 1 (IGF-1) is a hormone that plays an important role in growth, development, and metabolism. Little is known about its ability to regulate immune function. This study sought to address whether IGF-1 plays an inflammatory or anti-inflammatory role during an immune response. Our findings suggest that an upregulation of its primary receptor, IGF-1R, is indicative of an inflammation. Blood was drawn from 16 dogs and analyzed via flow cytometry for proteins consistent with an inflammatory phenotype. Samples from 10 dogs were stained with anti-CD11b, anti-CD14, and anti-IGF-1R. Samples from the remaining 6 dogs were stained for anti-CD14, anti-CD4, and IGF-1R directly bound to APC in 6 dogs. We suspect that using IGF-1 rather than its antibody will demonstrate a more physiological response, i.e. one more akin to what would take place in the dog. We found that expression of CD14, a co-receptor for TLR's that is widely used as a marker for monocytes, is upregulated as IGF-1R levels increase, suggesting that the number of canine monocytes increases as IGF-1R levels do. We then asked if a particular subpopulation of monocytes upregulated IGF-1R at higher rates. Using a gating scheme designed by Sampath Kumar and Alex Crane, we distinguished what we believe to be inflammatory classical monocytes from non-classical monocytes, which are thought to be imbued with suppressive function, using anti-CD4. Our IGF-1R stains suggest that a population of CD4- monocytes, i.e. classical monocytes, upregulate IGF-1R. This is consistent with our hypothesis that IGF-1R is associated with inflammation. Lastly, this study evaluated CD11b expression. CD11b is an integrin protein and myeloid marker that is correlated with activation status of an immune cell and can also be used as an inflammatory marker. We found that CD11b, like CD14, increases as IGF-1R expression increases, albeit at a smaller rate.

Background



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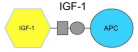
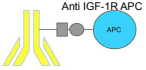
Insulin-like growth factor 1 (IGF-1) is a hormone that mediates growth and metabolism in many organisms, including dogs and humans. Its structural similarities to insulin allow the protein to bind both IGF-1 and insulin receptors. IGF1R is a tetrameric transmembrane receptor tyrosine kinase. Upon its activation, adapter proteins are recruited to the receptor and catalyze signaling cascades which result in cell proliferation, survival, transformation, metastasis, and angiogenesis (Huang X, 2015, 12-13).

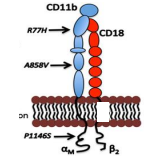
Fluorochrome-labeled recombinant IGF-1



Anti IGF-1R APC

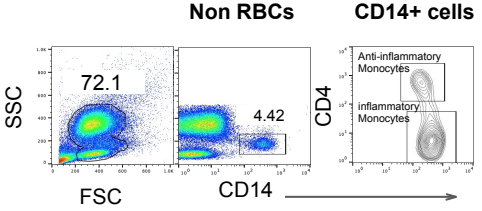


In our experiments, both human IGF-1 and anti-IGF-1R were bound to APC fluorophores via Lynx conjugation and used as markers for IGF-1R in canine blood samples. The IGF-1 protein was used as a marker in attempts to stain the cells more accurately, as it is possible that anti-IGF-1R binds Fc receptor as well as IGF-1R.



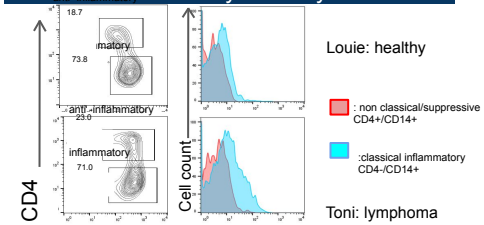
CD11b is part of the integrin family and binds with CD18 to form a membrane receptor known as CR3. This marker is expressed on numerous types of white blood cells, including monocytes. Although scientific literature contains conflicting information regarding its role in immune response, there is evidence to suggest that it mediates leukocyte response to infection, resulting in inflammation (Khan SQ, 2018, 2). Therefore, we decided to use CD11b as a marker for an inflammatory phenotype when analyzing canine monocytes.

Identifying Monocytes in Peripheral Blood



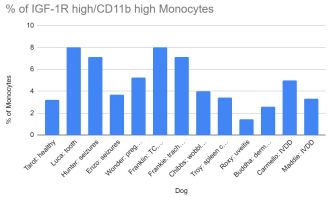
Gating strategy for identifying canine monocyte populations via FACS sorting. Anti-CD4-PE and anti-CD14-PE-Cy7 were incubated with whole blood. CD4+/CD14+ monocyte populations are thought to be suppressive, and CD4-/CD14++ monocytes are believed to be inflammatory.

Increased IGF-1R Expression in Inflammatory Monocytes



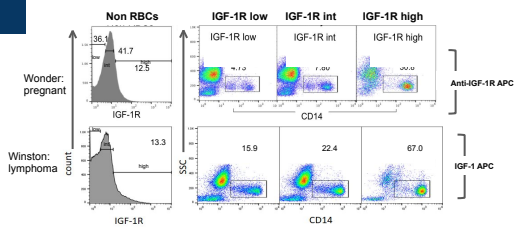
Higher expression levels of IGF-1R in inflammatory classical canine monocytes. Using the gating strategy previously described, monocytes from two dogs were isolated and divided into two subpopulations based on CD4 expression. This was measured by anti-CD4 PE. These CD14+, CD4+ and CD14+, CD4- cells were then analyzed for IGF-1R expression using IGF-1 bound to APC.

Is there a correlation between illness and CD11b/IGF1R expression in canine monocytes?

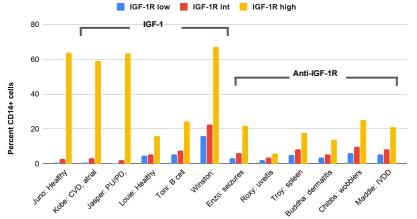


No significant correlation was found between illness and CD11b/IGF-1R expression in canine monocytes. Using the aforementioned gating strategy monocytes from 13 dogs, all varying in health, were sorted and analyzed for CD11b and IGF-1R expression using APC fluorophores bound to antibodies specific for those markers.

CD14 Upregulation with Increased IGF-1R Levels in Canine Monocytes



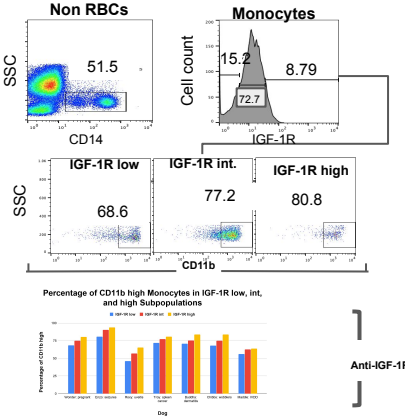
Percentage of CD14+ cells in IGF-1R low, int, and high non-RBCs



Percentage of monocytes increases as IGF-1R expression increases. Anti-IGF-1R-APC (top plots) and IGF1-APC (bottom plots) were used to determine the levels of IGF-1R expression in canine white blood cells. Both methods exhibit CD14 upregulation with increased IGF-1R levels in canine monocytes. Non RBCs in these subpopulations were isolated using the aforementioned gating strategy.

CD11b Upregulation with Increased IGF-1R Levels in Canine Monocytes

We then asked if any other inflammatory molecules were correlated with IGF-1R expression. We measured expression of CD11b, an integrin protein and indicator of activation status in myeloid cells.



CD11b expression is correlated with IGF-1R levels in canine monocytes. Fluorescently-labeled anti-IGF-1R was used. The percentages of CD11b high cells in IGF-1R low, int, and high populations were plotted on a graph and analyzed. CD11b levels increase as IGF-1R levels increase. Monocyte populations were isolated using the aforementioned gating strategy.

Concluding Remarks

This study found that :

-CD14 upregulation is associated with increasing IGF-1R levels
-IGF-1R expression was higher in CD4-CD14+ cells, i.e. classical inflammatory monocytes than in CD4+CD14+ cells, i.e. nonclassical suppressive monocytes
-IGF-1R expression is correlated with CD11b levels
-The correlation between IGF-1R and these markers suggests that high IGF-1R expression is consistent with an inflammatory phenotype in canine monocytes.
-We were unable to find a correlation between illness and IGF-1R expression, but that may not be representative of what would take place physiologically in the dogs' blood.

Directions for Further Research

Directions for further research include repeating the experiments after stimulation in an effort to replicate the conditions of an immune response. We also plan to eliminate unnecessary variables, e.g. use only IGF-1 APC rather than the antibody, as well as consider the dogs' varying medications.

References

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