Daily Variation in Monocyte Subsets and Toll-like Receptor 2 and 4 Expression in Young and Old mice.

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BACKGROUND: Aging is associated with an increase in systemic inflammation and chronic diseases; immune cells, such as monocytes, have been assessed in order to determine the state of the immune system. While aging research is important, longitudinal aging studies in humans can be time-consuming and impractical. Mouse models are ideal for aging research because of their short lifespan and the ability to highly control their environmental conditions. Current aging literature using mouse models consists mostly of cross-sectional and endpoint studies. Our laboratory utilizes a non-lethal blood draw technique, used monthly or weekly, to assess longitudinal alterations in blood monocytes and their surface proteins. Specifically, our lab has focused on exploring the role of Toll-like receptors 2 and 4 in age-related changes in inflammation and immune function. Due to their short half-life (1-3 days), it is necessary to understand daily variability in measurement outcomes in order to support the use of weekly or monthly assessments in our longitudinal model.

PURPOSE: The purpose of this study was to investigate the daily variation in peripheral blood monocyte subsets and TLR2/4 expression over a 5 day period in young and old mice. A second purpose was to confirm that differences between groups were consistent for the duration of the study, despite any changes due to daily variation.

METHODS: Blood was collected from a group of young (Y, N=9) and old (O, N=5) mice for five consecutive days. Flow cytometry was used to identify monocytes (Cd115+ events), to distinguish non-classic (CD115+/Ly6Cdim) and classic (CD115+/Ly6Cbright) subsets and to quantify TLR2 and TLR4 expression on the total monocyte population and each subset. Statistical analysis was completed using a 2 (groups: Y, O) x 5 (timepoint: Day 1, 2, 3, 4, 5) repeated measures ANOVA, with repeated measures on the second factor. Significant differences were located using a Tukey post hoc test.

RESULTS: Both young and old mice had significant fluctuations in total monocyte concentration, as well as in the proportions of the classic and non-classic subsets (P<0.05). Old mice had significant higher monocyte concentration than young mice at day 1, day 3 and day 4 (P<0.05). Old mice had a higher proportion of classic monocytes and lower proportion of non-classic monocytes on day 1, day 4, and day 5 (P<0.05). A significant difference in both TLR2 and TLR4 expression existed at all time points between the classic and non-classic monocytes in both groups, with classic expressing more of both (P<0.05). No significant group differences in TLR2 and TLR4 expression existed within either monocyte subset.
**Figure 1.** Over the 5-day study, fluctuations were seen in A) monocyte concentration, B) proportion of monocytes that are the classic subset, and C) proportion of monocytes that are the non-classic subset. * denotes a significant difference between groups (P<.05).

**CONCLUSION:** The increased monocyte concentration and greater proportion of the classic subset seen in the old mice may be implicated in the increase in systemic inflammation associated with aging. The increased TLR2 and TLR4 expression on the classic monocytes also likely contributes to this inflammation, since TRL2 and TLR4 are mediators of inflammation. Peripheral blood monocytes appear to fluctuate significantly over a 5-day period. Since differences between groups did not remain consistent throughout the study, these fluctuations may affect weekly or monthly measurements of monocyte concentration or monocyte subset proportions.