

Age-Related Differences in Monocyte Toll-Like Receptor 2/4 and CD54 Expression in Mice

Strohacker K, Breslin WL, McFarlin BK

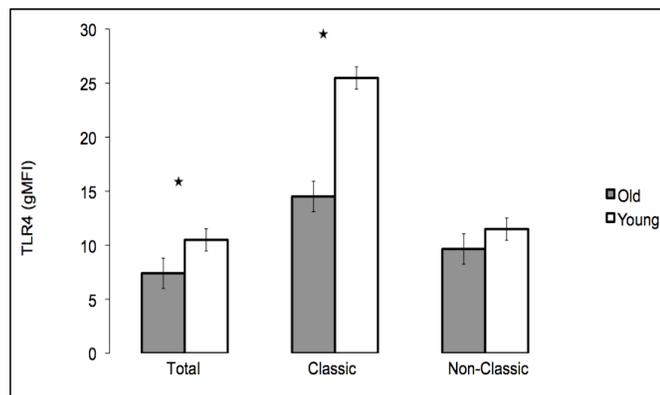
University of Houston

BACKGROUND: Aging is associated with immune dysfunction, such that chronic inflammation or immunosuppression can increase onset of disease and morbidity [1]. Health of the immune system has been assessed by measuring monocytes and their protein expression. While it has been shown in humans that advanced age is associated with higher proportions of pro-inflammatory monocytes and alteration in monocyte expression of toll-like receptors and adhesion molecules, no such monocyte data exists in mice [2]. Mouse models are ideal for aging research and are prevalent in published literature. Furthermore, mice have homologous inflammatory (classic) and resident (non-classic) monocytes. Therefore, it is important to determine if the expression of monocyte proteins differ between subsets and between age groups in mice.

PURPOSE: The purpose of this study was to determine age-related differences in toll-like receptor and adhesion molecule expression on mouse monocyte subsets.

METHODS: Two groups of outbred, CD-1 male mice were used; young (N=12, age 20 weeks) mice were compared to old (N=5, age 80 weeks) for this cross-sectional analysis. Young mice consumed a stock diet (13.5% fat) and old mice consumed a low-fat diet (10% fat) *ad libitum* since 12 weeks of age. Blood was drawn from the saphenous vein using a non-lethal technique. 3-color flow cytometry was used to assess percent of monocytes (CD115⁺), percent of monocytes that were classic (CD115⁺/Gr-1⁺) and non-classic (CD115⁺/Gr-1⁻) and cell surface expression of TLR2, TLR4 and CD54 (intracellular adhesion molecule) on each of subsets. Independent T-tests were used to determine statistically significant differences between group means.

RESULTS: Monocyte concentration 62% lower in young mice; 4% of cells in young mice were monocytes versus the 11% of monocytes in old mice. Old mice also had 40% more classic monocytes and 11% less non-classic monocytes compared to young mice ($P < 0.05$). There was no age difference in cell surface expression of TLR2 on either monocyte subset. However, classic TLR2 expression was 27% and 29% greater than non-classic expression in old and young mice, respectively ($P < 0.05$). TLR4 expression in the classic subset was 75% greater than in old mice. In young mice, classic TLR4 expression was 55% higher than in non-classic ($P < 0.05$). In old mice, classic TLR4



expression was 55% higher than in non-classic ($P < 0.05$). In old mice, classic TLR4

expression was only 34% higher than in non-classic, which was not statistically significant. There was no significant difference in CD54 expression, either between age groups or between monocyte subsets.

CONCLUSION: Proportions of total monocytes and of the classic (inflammatory) subset were increased in old mice, which is consistent with findings of previous research suggesting aging is associated with systemic inflammation [2]. Lower expression of TLR4 on the classic subset may hinder cytokine production. Reduced TLR4 is consistent with findings suggesting that macrophages of aged mice have reduced toll-like receptor expression and impaired cytokine production [3]. Therefore aged mice, like humans, may experience immunosuppression that can increase risk of infection. TLR2 induces a pro-inflammatory response when stimulated by gram-positive bacteria. This receptor can be stimulated to upregulate by glucose [5], which is often elevated with age and can further initiate pro-inflammatory effects. Research in elderly humans suggests that adhesion molecules are impaired with age [4]; while we observed no statistically significant difference, old mice tended to have greater expression of TLR2 and CD54. The lack of significance may be due to the small sample number. The results of this pilot study warrant further projects aimed at understanding age-related alterations in mouse monocytes.

REFERENCES:

- [1] Licastro F, Candore G, Lio D, Porcellini E, Colonna-Romano G, et al. 2005. Innate immunity and inflammation in ageing: a key for understanding age-related diseases. *Immun Ageing* 2: 8
- [2] Sadeghi HM, Schnelle JF, Thoma JK, Nishanian P, Fahey JL. 1999. Phenotypic and functional characteristics of circulating monocytes of elderly persons. *Exp Gerontol* 34: 959-70
- [3] Renshaw M, Rockwell J, Engleman C, Gewirtz A, Katz J, et al. 2002. Cutting Edge: Impaired toll-like receptor expression and function in aging. *J Immunol* 169: 4697-4701
- [4] De Martinis M, Modesti M, Ginaldi L 2004. Phenotypic and functional changes of circulating monocytes and polymorphonuclear leucocytes from elderly persons. *Immunol and Cell Biol* 82: 415-420
- [5] Devaraj S, Dasu MR, Rockwood J, Winter W, Griffen SC, Jialal I. 2008. Increased toll-like receptor (TLR) 2 and TLR4 expression in monocytes from patients with type 1 diabetes: further evidence of a proinflammatory state. *J Clin Endocrinol Metab* 93: 578-83