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NEUTROPHILS

Growing old disgracefully?

Although macrophages are appreciated to be highly heterogeneous, less attention has been paid to the heterogeneity that exists among neutrophil populations. Zhang *et al.* now report that neutrophils that have aged in the circulation show increased pro-inflammatory activity compared with neutrophils that are newly released from the bone marrow. Furthermore, they show that this enhancement of pro-inflammatory activity in ageing neutrophils is driven by the microbiota.

Previous studies examining neutrophil ageing in sterile *in vitro* systems had suggested that neutrophils become less inflammatory and show impaired migratory potential with age. In adoptive transfer studies in which neutrophils were aged in the circulation for up to 9 hours, Zhang *et al.* confirmed that neutrophils lose expression of the selectin CD62L as they age. However, when they analysed neutrophils adhering to tumour necrosis factor (TNF)-activated post-capillary venules, they found that aged $CD62L^{low}$ neutrophils showed enhanced activation of αMβ2 integrin.

To assess whether this was related to ageing or inflammation, the authors analysed the transcriptomes of aged neutrophils (assessed after 6 hours in the circulation), activated neutrophils (isolated from TNF-treated mice) and control neutrophils (assessed after 10 minutes in the circulation). Gene-set enrichment analyses identified several pathways that are upregulated by both aged and activated neutrophils, including pathways related to Toll-like receptor (TLR), NOD-like receptor and nuclear factor-κB signalling as well as to cell adhesion. Moreover, mice in which the numbers of aged neutrophils had been enriched (either by treatment with P-selectin- and E-selectin-specific blocking antibodies or through depletion of macrophages) showed enhanced production of reactive oxygen species and increased formation of neutrophil extracellular traps (NETs). Therefore, in contrast to what has been previously reported, neutrophils seem to show increased pro-inflammatory activity as they age.

> “enhancement of pro-inflammatory activity in ageing neutrophils is driven by the microbiota”

Further analyses indicated that neutrophils receive constitutive priming signals in the circulation that promote ageing, and the authors suspected involvement of the microbiota. In keeping with this, treatment of mice with antibiotics reduced both the numbers and overall percentages of aged neutrophils in the circulation, but this was reversed if mice were treated with TLR2 or TLR4 ligands. Experiments in which neutrophils were transferred into control, antibiotic-treated or germ-free recipient mice also suggested that neutrophil ageing is driven by the microbiota.

The authors showed that signalling via TLR2, TLR4 and the TLR adaptor MYD88 contributes to neutrophil ageing, and neutrophil ageing was almost completely abrogated when MYD88-deficient neutrophils were transferred into wild-type recipients. In a lipopolysaccharide-induced model of septic shock, they found that NET formation and fibrin deposition were reduced in the livers of mice that had been treated with antibiotics, and these mice showed prolonged survival compared with controls. The authors also identified a marked expansion of aged neutrophils in the circulation of mice with sickle cell disease (SCD), a model in which pathology is driven by neutrophil-mediated vaso-occlusion. Notably, antibiotic treatment decreased disease symptoms and improved survival in SCD mice. By contrast, depletion of macrophages in SCD mice led to an increase in the numbers of circulating aged neutrophils and promoted rapid death in these animals.

Finally, the authors compared neutrophil populations in patients with SCD and healthy controls. They identified no difference in overall neutrophil numbers between these groups but found that patients with SCD had increased numbers of aged neutrophils in the circulation. Notably, patients with SCD who had been administered penicillin V as a prophylactic therapy showed a decrease in both numbers and proportions of aged circulating neutrophils compared with untreated patients.

These data suggest that the microbiota drives ageing and the acquisition of pro-inflammatory functions in circulating neutrophils. As such, manipulation of the microbiota may have therapeutic potential by regulating disease-promoting subsets of neutrophils.

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ORIGINAL RESEARCH PAPER Zhang, D. *et al.* Neutrophil ageing is regulated by the microbiome. *Nature 525, 528–532 (2015)*