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Neutrophil Response to Cyclophosphamide Predicts Resilience to Age-related Learning Impairment

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Abstract

Ability to respond to stress, defined as resilience, was measured by white blood cell counts in various age C57BL/6 mice receiving a nonlethal dose of cyclophosphamide (CYP). Neutrophil counts dipped and then rebounded in a consistent and age-dependent manner. Low neutrophil rebound correlated with improved learning in middle age mice suggesting CYP-induced neutrophil response may predict resilience to aging.

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Introduction

Resilience is the ability to respond and recover from stressors which disrupt homeostasis. Cyclophosphamide (CYP) is a chemotherapeutic drug that halts cell cycling of precursor neutrophils, which decreases the number of mature neutrophils until the drug is eliminated from body. In this study, CYP was used as a stressor, where the resilience of mice to respond to one dose was quantified by changes in circulating neutrophil percentages and compared to the aging parameter of learning ability.

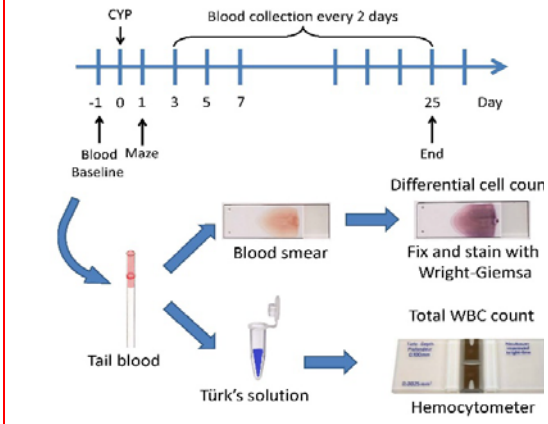

Figure 1. Timeline of experiment and outline of white blood cell assessment procedures.

Methods

C57Bl/6 male mice aged 4, 12, 17, and 28 months old were injected with 100mg/kg CYP intraperitoneally. Control groups were injected with 0.9% saline. A drop of tail blood was collected periodically after injection and white blood cells were counted (Figure 1). A Box Maze was used to assess learning ability (Figure 2). An n of 8-10 mice were used per age cohort.

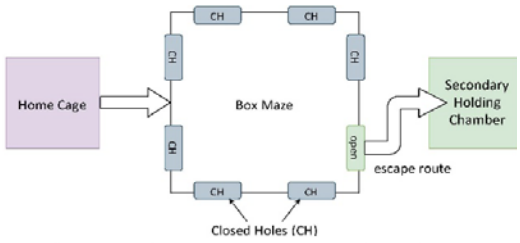

Figure 2. Box maze learning paradigm. Five 2-minute trials were conducted per mouse, noting time to reach open escape hole.

Results and Discussion

The neutrophil percentages followed a predictable pattern across age groups: an initial increase after injection, then a decrease reaching a nadir at day 5, and a rebound at day 7 (Figures 3A, 3B, 3C, and 3D). The degree to which neutrophil percentages changed was dependent upon age; in particular, the strength of the neutrophil percentage rebound from day 5 to day 7 decreased with increasing age (Figure 4A). Neutrophil rebound negatively correlated with escape speed from a box maze, a learning paradigm where higher speeds are associated with resilience to age-related cognitive decline (Figure 4B).

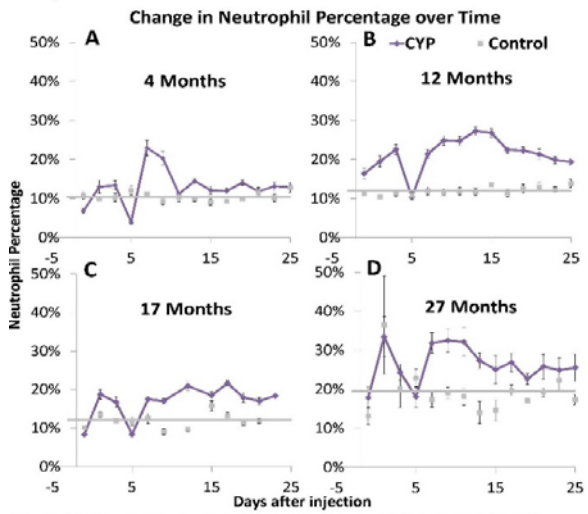

Figure 3. Change in neutrophil percentage relative to total white blood cells for both control and CYP treated mice over a period of 27 days. Mice separated by ages into **(A)** 4 months, **(B)** 12 months, **(C)** 17 months, and **(D)** 27 months.

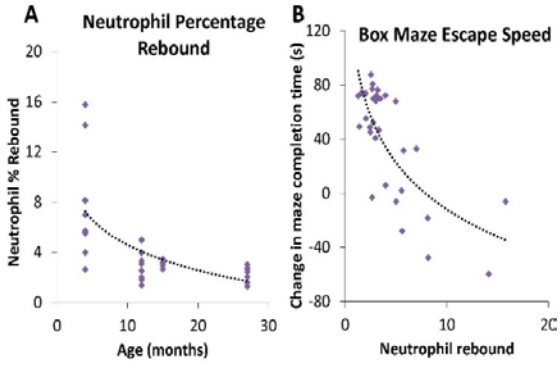

Figure 4. (A) As age of mice increased, the strength of the neutrophil rebound decreased. **(B)** The change in box maze escape time from trial 1 to trial 5 was negatively correlated with neutrophil rebound response.

One dose of CYP induced a neutrophil rebound response that was consistent over all age groups. The rebound strength decreased with increasing age, so it was age dependent. Low CYP-induced neutrophil rebound response was associated with decreased learning impairment. This may predict resilience to aging given the correlation with established cognitive aging parameters, though the cause of this relationship requires further study. These observations provide the rationale for developing clinical *in vitro* investigations using CYP-induced stress in human peripheral white blood cells.