FASTING AND DIETARY RESTRICTION IMPACT ON IMMUNE SYSTEM IN AGING POPULATION: A NARRATIVE REVIEW

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Abstract
Dietary habits and nutrition metabolism are strongly associated with human physiology. Overnutrition or calorie excess has been shown to have many deleterious effects. At the same time, the impact of fasting as part of dietary restriction (DR) remains elusive, especially on the immune system during the aging process. Here, we describe current updates of fasting and DR impact on the immune system and host responses in the aging population. Generally, fasting causes alteration in immune cell distribution by increasing the homing of peripheral cells to the bone marrow. In the aging population, fasting reduces the inflammatory response and promotes cell regeneration. Additionally, fasting is associated with the B cell clonal expansions important to counter infection. This review offers new perspectives and enhances our understanding of immunosenescence as a key to healthy aging.

Keywords
Aging, Dietary Restriction, Fasting, Immunosenescence
Introduction

During aging, the immune system typically experiences functional dysregulation, leading to an increased susceptibility to viral and bacterial infection. This, in turn, results in a higher incidence of cancer and age-related disease. This phenomenon is referred to as immunosenescence. In the elderly population (those aged over 65 years), the accumulation of aging-related factors, such as the acquisition of senescence-associated secretory phenotype cells, macrophage activation, oxidative stress, adiposity, and gut dysbiosis, promotes a chronic inflammation that contributes to immunosenescence (Figure 1). Age-related impairments in hematopoietic stem cells (HSC) lead to a skewed differentiation toward a myeloid lineage, thereby decreasing common lymphoid progenitors, T- and B-cell lymphogenesis, and regenerative capacity. The reduction in naive lymphocytes and the expansion of late-differentiated and exhausted lymphocytes further impair immune function and may contribute to cancer and autoimmune disease.

Figure 1. Age-related changes in various immune cells.
Aging induces chronic inflammation responses due to the accumulation of immune dysfunction in the innate and adaptive immune systems.

Among the various factors associated with aging, nutrition has been shown to significantly impact the immune system. Dietary restriction (DR), a 20-40% reduction in daily calorie intake without causing malnutrition, both in the short term (less than four weeks) and the long-term (more than six months), is known to play a crucial role in extending life span and delaying many aging-related diseases, such as
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diabetes, atherosclerosis, cardiovascular disease, kidney disease, autoimmune disease and neurodegenerative conditions like Parkinson’s and Alzheimer disease. The prevention of many aging-related diseases by DR and fasting has been linked to immune responses, including its anti-inflammatory qualities, reduction in reactive oxygen species, slowing of thymic involution, and decreased production of lymphocytes.9

In this review, we discuss the effect of nutritional interventions, especially fasting and DR, on immunosenescence in the context of innate and adaptive immunity. To explore the clinical and molecular aspects of the topic, we have not limited our resources to human studies alone but have also included studies on animal models. This study broadens our perspectives and enhances our understanding of immunosenescence as a cornerstone for successful aging.

**Effect of fasting or DR on innate immunity during aging**

Innate immunity, which encompasses the body’s initial defense mechanism such as the skin, mucosal layer, and non-lymphocyte immune cells, plays a crucial role in mounting a rapid response to immune disturbances.10 In this section, we will focus on the effects of DR and fasting on age-related changes in innate immune cells, which are closely linked to inflammatory response.

As individuals age, the innate immune system activates an inflammatory network, and DR has been shown to reduce this response effectively.11 A small-scale clinical study conducted by Walrand et al.12 examined changes in peripheral blood and observed that fasting and subsequent refeeding increased the total blood neutrophil and white blood count, regardless of age. However, fasting decreased the neutrophil chemotaxis index in both young adults and elderly populations, and the migration capacity was restored by refeeding only in young adults but not in the elderly.12

Ma et al. used a mouse model to perform single-cell analyses, which revealed a notable increase in neutrophils in multiple tissues, except bone marrow, during aging. These neutrophil numbers decreased with DR. In the aging population, neutrophils may migrate from the bone marrow to infiltrate peripheral tissues such as brown adipose tissue (BAT), white adipose tissue (WAT), liver, and kidney tissues. DR may reverse this migration. Increased pro-inflammatory macrophage infiltration was observed in various aged tissues, but DR reversed this, mitigating chronic inflammation associated with aging-associated conditions.13 Similarly, Rasa et al. demonstrated that DR inhibits the age-induced dysregulation of pro-inflammatory cytokines, such as interferon (IFN) and tumor necrotizing factors (TNF).11

Janssen et al. used animal models to comprehensively analyze the distribution of immune cells during fasting, focusing not exclusively on aging. Fasting leads to the reorganization of the leucocyte landscape across various organs. They observed the
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Reentry of monocytes into bone marrow during fasting. Refeeding after prolonged fasting results in a surge of monocytes in circulation, which alters the immune response to bacterial infections. Another study by Tao Si et al. reported that DR significantly corrects the imbalance in the HSC pool in older mice (15-18 months of age, equivalent to 50-60 years in humans), particularly the myeloid-biased HSCs (CD150^high HSC and CD41^HSC) which subsequently develop into non-specific immune cells (neutrophils, monocytes, macrophages). In contrast, the lymphoid-biased HSCs (CD150^low HSC and CD41^HSC) which later develop into the adaptive immune system (B and T cell lymphocytes), remain unaltered. HSC from DR mice exhibited increased quiescence, preventing the accumulation of DNA damage and functional decline in HSC. Long-term DR restored the expression of mitochondrial Unfolded Protein Response (UPRmt) genes (C1pP, HSP10, and HSP60) in aging HSC, along with the UPRmt upstream suppressor Sirtuin 7 (SIRT7) and the SIRT2-NLRP3-caspase 1 axis to levels comparable to young HSCs. Targeting the SIRT2-NLRP3-caspase 1 axis is known to be able to reverse the age-related functional deterioration of HSC.

Another population of innate immune cells influenced by fasting and aging is natural killer (NK) cells, the predominant innate lymphocytes mediating antitumor and antiviral responses. A study by White et al. demonstrated an association between DR and the accumulation of immature/early-stage NK cells. They also reported reduced expression of Ly49 receptors in calorie-restricted mice, which is related to self-antigen recognition by NK cells. There was a tendency for increased NK proliferation in response to IL12/IL18 in calorie-restricted mice. However, the functional effect of calorie restriction on NK physiology is yet to be fully elucidated.

**The adaptive immune response of the aging population during fasting or DR**

The adaptive immune system, which includes the B and T cells (lymphocytes) activation, works synergistically with innate immunity to defend against immune perturbations. Adaptive immune cells provide a specific memory capacity for rapid pathogen elimination during secondary infection.

B cells development occurs in the bone marrow, where lymphoid progenitor cells begin to differentiate from pro-B cells, pre-B cells, immature B cells, to ultimately recirculating mature B cells. As previously mentioned, the decreased lymphopoiesis in the bone marrow during aging, particularly that of B cells, was not rescued by DR. Sushimita et al. reported that both DR and fasting in mice led to a significant reduction in the population of pro-B, pre-B, and immature-B cells in the bone marrow, accompanied by a significant increase in circulating mature B cells. Evidently, dietary changes prompt the bone marrow to conserve energy by inhibiting the generation of additional B cells while prioritizing energy preservation to manage metabolic...
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Imbalances. Fasting and DR also induced a depletion of immature transitional B cells (CD19⁺B220⁺IgM⁺IgD⁻) and mature B cell populations (CD19⁺B220⁺IgM⁺IgD⁺) in the spleen. Furthermore, fasting caused a significant depletion of the marginal zone and follicular B cells.

During aging, naive B cells are predominantly replaced by memory B cells, resulting in limited diversity of the B cell receptor repertoire and reduced antigen specificity. This lack of diversity is associated with a diminished capacity for antigen recognition in infections and vaccinations. The reduction in repertoire diversity is a direct consequence of clonal expansion among B cells.

DR initiated in mid-life mice (at 16 months of age) was sufficient to maintain both B cell receptor (BCR) repertoire diversity (evaluated using Shannon and Simpson diversity measures) and reduce clonal expansions in the spleen. DR mice exhibited an increased abundance of both IgM and IgG, a characteristic of a healthier BCR repertoire. Notably, there is an age-related increase in the production of autoantibodies, including IgG glycans, and the heightened IgG is essential for counteracting this phenomenon. Likewise, the rise in IgM levels may indicate an enhanced capacity for HSC regeneration and increased repertoire responsiveness.

Clonal diversity is crucial for effective antigen recognition. Upon encountering antigens in a T cell-dependent manner, naive B cells are prompted to enter germinal centers to generate high-affinity antibodies. This affinity maturation of B cells is achieved through class switch recombination (CSR) and somatic hypermutation (SHM). SHM introduces mutation into the BCR, resulting in increased affinity for antigens. Unlike the spleen, the BCR repertoire of the ileum showed only minor changes with aging and in response to DR. However, an increase in SHM suggests an enhanced capacity for antigen binding.

When it comes to T cells, White MJ et al. found no significant age-related changes in the proportion of CD4⁺, CD8⁺, or T regulatory among C57BL/6 mice in the spleen, lung, liver, and lymph nodes. However, DR significantly increased the proportion of CD8⁺ cells, leading to a corresponding decline in the CD4/CD8 ratio. Asami T et al. observed age-associated decreases in the proportions of CD4⁺ and CD8⁺ T cells, which were attenuated by long-term DR.

Aging reduces the proportion of naive (CD44⁻CD11a⁻) CD4⁺ and CD8⁺ cells, leading to a skewing of the T cell phenotype toward memory T cells. Remarkably, long-term DR significantly delayed the transition from a naive to effector T cell phenotype in aged mice, resulting in a distribution of naive/effector T cells that resembled that of young mice.

Furthermore, during aging, there is a decline in the generation of T cell cytokines such as interleukin (IL)-2, interferon, (IFN)-γ and granzyme B, all of which play pivotal roles in coordinating both innate and adaptive immune system responses. Long-term DR significantly increased the numbers of CD4⁺ T cells.
producing IL-2 and IFN-γ, as well as granzyme B-producing CD4+ and CD8+ T cells. However, it had no impact on the production of these cytokines by CD8+ T cells. Additionally, DR significantly reduced the transcription factors NR4A1 and TOX, leading to a decrease in exhausted T cells marked by KLRG1 and Tim3. Together, the effects of fasting and DR on the immune system in aging are illustrated in Figure 2.

**Figure 2.** Effect of fasting and DR in the immunosenescence. Fasting alters the distribution of immune cell and decrease inflammation in aging.

**Conclusion**

Dietary interventions have been proven to be effective in reducing major inflammatory disturbances associated with aging-related immune perturbations and in suppressing organ damage and inflammation. In summary, fasting and DR can alter immune cell distribution. However, further studies, including humans, are needed to gain a more comprehensive understanding of the molecular mechanism and clinical impact of fasting to immunosenescence in humans.

**Conflict of Interests**

The authors declare no conflict of interest.
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