

Cold stress-regulated immune responses: Insights, challenges, and perspectives

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1 Cold stress reshapes the immune status

The winter of 2019 marked history with the advent of the COVID-19 Coronavirus pandemic; Cross-country skiers are more susceptible to asthmatic symptoms than their peers in warmer places^[1]. The influenza virus causes a more severe infection in winter than in warmer seasons^[2]. These phenomena suggest that exposure to cold weather increases a person's vulnerability to health concerns such as the aforementioned "colds" and asthma. While it is accepted that the term "cold" covers a wide array of ailments, it has long been questioned how cold weather brings forth these illnesses. The underlying mechanisms remain to be determined.

Cold temperature refers to a temperature lower than 12°C in the air or below 20°C in water^[3]. Exposure to these low temperatures can induce physiological changes in humans and animals such as cellular and molecular adaptations that provide a defense against cold-induced damages. Cold temperatures increase mucus viscosity and decrease ciliary action in the upper respiratory system^[1]. Very cold temperatures, lower than 0°C, can damage normal physical barriers and impair normal immune functions, resulting in greater susceptibility to infection from pathogens. Exposure to cold temperature reduces lymphoproliferation but increases stress hormones such as corticosteroids, catecholamines, epinephrine, norepinephrine, cortisol, and aldosterone^[3]. The increase of these hormones results in leukocytosis and suppresses the production of inflammatory factors and adhesion molecules, thereby altering the immune system^[3]. Therefore, people that reside in cold environments have greater levels of stress-induced immune impairment compared to peers that live in mild climates. Besides hormone secretion, cold temperature also causes cellular remodeling in humans. The most notable cell type affected by cold temperature is the Natural-killer (NK) cell, a critical innate immune cytotoxic effector. The ability of NK cells to kill infected or cancerous cells, termed

NK cell activity (NKCA), is recognized as an index of innate immune functions^[4]. Furthermore, NK cells also direct immune responses through the production of Type 1 and Type 2 cytokines. Hence, decreased levels of NK cells after exposure to cold temperatures predisposes the host to infections.

2 Mechanisms underlying cold regulated immune responses

Cold stress not only regulates the innate but also the adaptive immune system. First, exposure to suboptimal temperatures redirects energy usage towards the generation of heat, thereby decreasing the energy used by the immune system^[5]. Second, low-temperature exposure can reduce plasma proteins and enzymes that regulate membrane transport activities and skeleton mineralization^[6]. As revealed in pufferfish, cold stress decreases total blood cell count, impairs cell viability, and subsequently leads to DNA damage^[6]. Third, the phagocytic index of blood leukocytes isolated from various types of fish is reduced after cold exposure^[7]. Evidence obtained in fish demonstrates that the genes encoding antioxidant enzymes, such as *SOD*, *CAT*, *HSP90*, and *C3*, and genes related apoptosis, such as *P53*, *caspase-9*, and *caspase-3*, are increased in exposure to cold temperatures^[6]. Cold exposure also improves the production of pro-inflammatory cytokines such as *TNF α* , *IFN γ* , *IL-1 β* , and *IL-6*^[5]. As revealed in fish exposed to cold stress, genes of crucial proinflammatory cytokines such as *il1b*, *tnfa*, *ifn1*, *ifng*, *inos*, *irf3*, and *mda5*, are suppressed^[7]. These cytokines are critical contributors to viral recognition, which suggests that cold stress can make hosts vulnerable to viral infections.

Leukocyte cellularity in the major immune organs, such as blood, kidney, lymph nodes, and spleen is suppressed by cold stress, as revealed by rainbow trout challenged with bacterial and protozoal pathogens^[7]. This is in part because cold stress inhibits the proliferation and activations of T and

B lymphocytes^[7]. Additionally, cold exposure boosts up the generation of myeloid-derived suppressor cells (MDSCs) that suppress T cell proliferation^[6]. Through the ASK1-p38 MAPK signaling pathway, severe cold stress induces ferroptosis, a type of regulated necrosis that is triggered by a combination of iron toxicity, lipid peroxidation, and plasma membrane damage^[8]. Moreover, in rainbow trout exposed to *Tetracapsuloides bryosalmona*, cold stress also affects the CD4 T cell differentiation towards Th1 cells, a CD4 T cell subset that stimulates cell-mediated immune responses, typically against intracellular bacteria and protozoa^[9]. Furthermore, as observed in channel catfish and carp, antibody production in B cells is proportional to temperature, demonstrating that the antibody production against pathogens is generally suppressed under cold stress^[7]. Additional studies showed that cold stress also influences antigen presentation in fish. For example, in carp, cell surface MHC I was downregulated upon exposure to cold temperatures^[7]. These results indicate that cold temperatures exert fine influences on lymphocytes, which in turn affects the adaptive immune responses.

3 Current challenges

Current studies on cold temperature regulated immune response face various challenges including a dearth of relevant data, suboptimal experimental design, inappropriate research models, contradictory results.

3.1 Limited studies

Only a small number of investigations focusing on cold temperature-regulated immune responses are available in recent years. The published studies predominantly focused on immune cells, hormones, and cytokines in peripheral blood. Many primary immune cells in lymphoid organs, such as T cells, B lymphocytes, dendritic cells, and their subsets that are dynamically involved in innate and adaptive immune responses have not been extensively explored. The temperatures tested (4°C-9°C)^[3] in many studies are not low enough and few studies have investigated temperatures below 0°C. Thus, the data obtained and conclusions reached may not be readily to extrapolated to the real-world situations.

3.2 Contradictory results

There have been conflicting reports on the magnitude and direction of cold stress-regulated immune responses in the literature. For example, some reports showed cold air at 4°C to 8°C increases NKCA, but others reported that 5°C air has no effect^[3]. One study demonstrated that low temperatures downregulate splenic antigen-binding, alter NK cell numbers and

cytotoxicity, and increase adrenal cortisol concentration, viremia, and neuro invasiveness of arboviruses^[3]. However, another work showed that rats housed at 5°C for 7 weeks secrete more IL-6 and TNF- α accompanied by increased macrophage and T-cell infiltration in kidneys compared to rats maintained under normal control conditions^[10]. This cytokine remodeling indicates stimulated immunity. The contradictions suggest that immune responses to cold weather are case-dependent. Even in the same species, lack of a fixed dose control is a notable factor for the observed differences because immunological responses depend on the severity (*i.e.*, time, accurate temperature) of the cold exposure.

3.3 Noise exclusion

In most circumstances, it is difficult to figure out whether a symptom is caused by exposure to cold temperatures or by other factors. For instance, cold temperature may cause symptoms similar to bacterial infections, such as bronchospasms and rhinitis^[11]. Furthermore, simultaneous exposure to both cold temperature and a bacterial infection can cause greater immune impairment than exposure to one factor alone. Such an additive or synergistic mechanism is reflected by the phenomenon that influenza virus infection is more severe in winter seasons. For some microorganisms, cold air is favorable for their proliferation. Whether cold is the determining or an additional factor in these circumstances requires more explorations with noise-canceling experimental designs.

3.4 Adaptation

During long-time or habitual cold exposure, hosts may develop adaptive mechanisms in their physiological or immune systems. For instance, in the blood of regular winter swimmers, the concentrations of plasma IL-6, monocytes, and leukocytes are remarkably higher than that of inexperienced swimmers^[12]. This highlights the need for more rational experimental design with reasonable duration and degree of cold exposure for more accurate examination of the influence of cold stress.

4 Perspective on further investigations

Cold environment practitioners, including winter athletes, swimmers, military personnel, and other people who have to work in cold conditions, face risks to their immune system and susceptibility to infections. Revealing the mechanism by which the cold exposure affects the human immune system is of profound importance. Though some progress has been achieved, various challenges still hinder the application of the results to humans. Generally speaking, more talented scientists

and larger scales of well-designed investigations are necessary and will make great contributions to this field. More specifically, diverse experimental models, relevant cold temperatures, sufficient exposure duration, and noise-canceling experimental settings are critical factors for conducting good investigations. Deeper exploration into immune responses against cold exposure will enrich our understanding of the mechanisms of immune dysfunction in cold environments, thereby improving adaptations to cold. Most of the published studies are descriptive with correlative and suggestive results. To uncover the causal factors underlying the effects of cold exposure on immune system, advanced genome analysis is an efficient approach for screening the genes determining or regulating cold-regulated immune responses. For elucidating the pathophysiological roles of cold-regulated immune responses, specific gene knock-out animal models are of great values. Translation of the experimental conclusions obtained from mice to humans is a big challenge faced by the scientific community. The major metabolic differences between humans and rodents is a critical factor limiting the direct extrapolation and applications

of animal data to humans^[6]. Thermoneutrality is the metabolic state of an organism in an environmental temperature at which it does not need generate or lose heat^[6]. Human physiology is maintained at temperatures within the thermoneutral zone (usually 29°C-34°C), but laboratory rodents are housed below this temperature (usually 20°C-22°C) and hence need more energy to generate heat^[6]. Of note, animals and humans have different tolerance to cold environments, creating a gap between different species. For translational medicine, the difference of physiological characteristics between animals and humans should be considered. To address this issue, lessons learned from transplantation will be helpful. That is, transition from mice to primates that are closer to humans in genomics and physiology, and then to humans, is the way to go. Last but not least, worldwide collaboration and willingness to share the scientific ideas and databases are critical to speed up the progress.

Conflicts of interests

The authors have declared that no conflicts of interests exists.

References

- [1] Sue-Chu M, Sandsund M, Helgerud J, *et al.* Salmeterol and physical performance at -15°C in highly trained nonasthmatic cross-country skiers. *Scand J Med Sci Sport*, 1999; 9(1): 48-52.
- [2] Dowell S F. Seasonal variation in host susceptibility and cycles of certain infectious diseases. *Emerg Infect Dis*, 2001; 7(3): 369-374.
- [3] LaVoy E C P, McFarlin B K, Simpson R J. Immune responses to exercising in a cold environment. *Wilderness Environ Med*, 2011; 22(4): 343-351.
- [4] Lee K J, An K O. Impact of high-intensity circuit resistance exercise on physical fitness, inflammation, and immune cells in female breast cancer survivors: a randomized control trial. *Int J Environ Res Public Health*, 2022; 19(9): 5463.
- [5] Vialard F, Olivier M. Thermoneutrality and immunity: how does cold stress affect disease? *Front. Immunol*, 2020; 11: 588387.
- [6] Cheng C H, Ye C X, Guo Z X, *et al.* Immune and physiological responses of pufferfish (*Takifugu obscurus*) under cold stress. *Fish & Shellfish Immunol*, 2017; 64: 137-145.
- [7] Abram Q H, Dixon B, Katzenback B A. Impacts of low temperature on the teleost immune system. *Biology (Basel)*, 2017; 6(4): 39.
- [8] Hattori K, Ishikawa H, Sakauchi C, *et al.* Cold stress-induced ferroptosis involves the ASK 1-p38 pathway. *EMBO Rep*, 2017; 18(11): 2067-2078.
- [9] Li L, Wu J, Abdi R, *et al.* Lymph node fibroblastic reticular cells steer immune responses. *Trends Immunol*, 2021; 42(8): 723-734.
- [10] Crosswhite P, Sun Z. Ribonucleic acid interference knockdown of interleukin 6 attenuates cold-induced hypertension. *Hypertension*, 2010; 55(6): 1484-1491.
- [11] Silvers W S. The skier's nose: a model of cold-induced rhinorrhea. *Ann Allergy*, 1991; 67(1): 32-36.
- [12] Dugué B, Leppanen E. Adaptation related to cytokines in man: effects of regular swimming in ice-cold water. *Clin Physiol*, 2000; 20(2): 114-121.