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IL-9 aggravates SARS-CoV-2 infection and exacerbates associated airway inflammation

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SARS-CoV-2 infection is known for causing broncho-alveolar inflammation. Interleukin 9 (IL-9) induces airway inflammation and bronchial hyper responsiveness in respiratory viral illnesses and allergic inflammation, however, IL-9 has not been assigned a pathologic role in COVID-19. Here we show, in a K18-hACE2 transgenic (ACE2.Tg) mouse model, that IL-9 contributes to and exacerbates viral spread and airway inflammation caused by SARS-CoV-2 infection. *ACE2.Tg* mice with $CD4^+$ T cell-specific deficiency of the transcription factor Forkhead Box Protein O1 (Foxo1) produce significantly less IL-9 upon SARS-CoV-2 infection than the wild type controls and they are resistant to the severe inflammatory disease that characterises the control mice. Exogenous IL-9 increases airway inflammation in Foxo1-deficient mice, while IL-9 blockade reduces and suppresses airway inflammation in SARS-CoV-2 infection, providing further evidence for a *Foxo1*-Il-9 mediated Th cell-specific pathway playing a role in COVID-19. Collectively, our study provides mechanistic insight into an important inflammatory pathway in SARS-CoV-2 infection, and thus represents proof of principle for the development of host-directed therapeutics to mitigate disease severity.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19). COVID-19 symptoms range from mild to severe pneumonia and acute respiratory distress syndrome[1]. SARS-CoV-2 infection leads to hyperactivation of immune cells, which further induces inflammatory cascade, broncho-alveolar inflammation and immunopathology[2]. Use of dexamethasone, an anti-inflammatory drug, resulted in lower mortality and severity in patients hospitalized with COVID-19[3], indicating that immune suppression is effective in controlling the severity and mortality. In addition, we identified that SARS-CoV-2 infection, in animal model, contributes to the extra-pulmonary pathologies which include cardiovascular complications and thymic atrophy[4].

Although the precise mechanism of disease pathogenesis and lung pathology that lead to hyper-inflammatory response is not fully understood, autopsy histopathology of pulmonary samples revealed increased accumulation of eosinophils, basophils, neutrophils and perivascular and septal mast cells in COVID-19[5–7]. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19 shows that one of the prominent cell types is Mast cells[7,8]. In line with this, mast cells-derived proteases, chymase and eosinophil-associated mediators are found to be elevated in sera of COVID-19 patient and lung autopsies[5,8]. Interleukin 9 (IL-9), a common γ chain family cytokine primarily produced by Th9 cells[9], promotes mast cell growth and function in allergic inflammation[10]. Although IL-9 plays an essential role in severe airway inflammation and bronchial hyper responsiveness in asthma and Respiratory Syncytial Virus (RSV) infection[11,12], the role of IL-9 is not yet identified in SARS-CoV-2 infection, and its associated immunopathology.

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IMMUNOLOGY

High-salt diet mediates interplay between NK cells and gut microbiota to induce potent tumor immunity

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High-salt diet (HSD) modulates effector and regulatory T cell functions and promotes tissue inflammation in autoimmune diseases. However, effects of HSD and its association with gut microbiota in tumor immunity remain undefined. Here, we report that HSD induces natural killer (NK) cell–mediated tumor immunity by inhibiting PD-1 expression while enhancing IFNγ and serum hippurate. Salt enhanced tumor immunity when combined with a suboptimal dose of anti-PD1 antibody. While HSD-induced tumor immunity was blunted upon gut microbiota depletion, fecal microbiota transplantation (FMT) from HSD mice restored the tumor immunity associated with NK cell functions. HSD increased the abundance of *Bifidobacterium* and caused increased gut permeability leading to intratumor localization of *Bifidobacterium*, which enhanced NK cell functions and tumor regression. Intratumoral injections of *Bifidobacterium* activated NK cells, which inhibited tumor growth. These results indicate that HSD modulates gut microbiome that induces NK cell–dependent tumor immunity with a potential translational perspective.

INTRODUCTION

Dietary components influence human health by regulating immune homeostasis and gut microbiota composition (*1*–*4*). Salt when taken in a higher amount [4% NaCl: high-salt diet (HSD)] has been identified as a potent immunomodulator associated with a strong inflammatory response (*5*–*7*). Recent studies identified that HSD exacerbates tissue inflammation in ulcerative colitis and autoimmune encephalomyelitis and increases the risk of cardiovascular diseases associated with enhanced T helper 17 (T_H17) cell development and functions (*5*, *8*, *9*). Other studies reported that HSD polarizes macrophages to M1-like phenotype and its association with elevated interferon-γ (IFNγ) response (*6*, *7*, *10*). A longitudinal study on healthy human participants found a strong correlation between HSD and monocyte frequency (*6*). On the basis of these observations, HSD could act as an inflammatory trigger that may overcome immunosuppressive conditions associated with tumor microenvironment such as the expression of checkpoint inhibitors and down-regulation of major histocompatibility complex I (MHC-I) molecules. Recent studies have shown that HSD can inhibit tumor growth, which may be dependent on myeloid-derived suppressor cells (MDSCs) (*11*, *12*).

Down-regulation of MHC-I is a strong activation signal for NK cell activation and mediates direct killing of tumor cells (*13*). Activation of NK cells is in turn controlled by a wide array of activation signals such as CD107a, natural cytotoxic trigger receptor 1 (NCR1), CD226, and inhibitory signals such as CD96, programmed cell death protein (PD) 1, T cell immunoglobulin and ITIM domain (TIGIT), T-cell immunoglobulin domain and mucin domain (Tim) 3, and cytotoxic T-lymphocyte associated protein (CTLA) 4 molecules (*14*–*17*). Furthermore, the tumor microenvironment is often characterized by ionic imbalance as decreased sodium level (hyponatremia) has been linked to human cancers (*18*, *19*). Altered Na^+/H^+ concentration across the gut epithelial barrier is linked with changes in gut permeability and dysbiosis, and previous reports have suggested that HSD induces changes in the gut microbiota composition and metabolic alterations in rodents (*20*). These shreds of evidence suggested that tumor immunity by HSD may involve factors from serum and gut microbiota and may influence other components of the immune system essential for antitumor functions.

In the current study, we report that tumor-bearing mice fed with HSD potently suppressed tumor growth by up-regulation of NK cell frequency and activation markers and down-regulation of NK cell inhibitory signals (especially PD1 molecule). NK cell depletion truncated the tumor immunity of HSD, which was found to be mediated by NK-dependent interferon-γ (IFNγ) response. We further establish that HSD, in mice, leads to marked up-regulation of serum hippurate, a microbial benzoate metabolism product that is also described as one of the metabolic markers of PD-1 immunotherapy in responding patients (*21*). In line with this, we report that the combination of a suboptimal dose of anti-PD1 antibody together with a low-salt diet provides a significant tumor regression. Antibiotic-induced gut microbiota depletion (AIMD) abrogated the HSD-mediated tumor inhibition and antitumor NK cell functions, indicating the involvement of gut microbiota. HSD-fed mice showed an increased abundance of *Bifidobacterium* in their stool, which upon transfer to AIMD mice, suppressed tumor progression associated with increased intratumor NK cell frequency and elevated serum hippurate levels. Last, we demonstrate that mice fed with HSD show an increased gut permeability resulted in intratumoral localization of *Bifidobacterium* leading to NK cell activation. While intratumoral administration of *Bifidobacterium* alone resulted in tumor regression, NK cell depletion blunted *Bifidobacterium*-mediated protection. Furthermore, increased hippurate levels were found in *Bifidobacterium* administered mice suggesting that hippurate might be a potential biomarker of HSD-mediated tumor immunity. Together,

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