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EGFR-HIF1α signaling positively regulates the differentiation of IL-9 producing T helper cells

Suyasha Roy[1], Zaigham Abbas Rizvi[1], Alexander J. Clarke[2], Felicity Macdonald[3], Abhaydeep Pandey[4], Dietmar Martin Werner Zaiss[3], Anna Kathrina Simon[2] & Amit Awasthi[1✉]

Interleukin 9 (IL-9)-producing helper T (Th9) cells are essential for inducing anti-tumor immunity and inflammation in allergic and autoimmune diseases. Although transcription factors that are essential for Th9 cell differentiation have been identified, other signaling pathways that are required for their generation and functions are yet to be explored. Here, we identify that Epidermal Growth Factor Receptor (EGFR) is essential for IL-9 induction in helper T (Th) cells. Moreover, amphiregulin (Areg), an EGFR ligand, is critical for the amplification of Th9 cells induced by TGF-β1 and IL-4. Furthermore, our data show that Areg-EGFR signaling induces HIF1α, which binds and transactivates IL-9 and NOS2 promoters in Th9 cells. Loss of EGFR or HIF1α abrogates Th9 cell differentiation and suppresses their anti-tumor functions. Moreover, in line with its reliance on HIF1α expression, metabolomics profiling of Th9 cells revealed that Succinate, a TCA cycle metabolite, promotes Th9 cell differentiation and Th9 cell-mediated tumor regression.

[1] Immuno-biology Laboratory, Translational Health Science & Technology Institute, Faridabad, Haryana, India. [2] Kennedy Institute of Rheumatology, University of Oxford, Oxford, UK. [3] Institute of Immunology and Infection Research, University of Edinburgh, Scotland, UK. [4] Translational Health Science & Technology Institute, Faridabad, Haryana, India. ✉email: aawasthi@thsti.res.in

IMMUNOLOGY

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

Zaigham Abbas Rizvi[1,2], Rajdeep Dalal[1,2], Srikanth Sadhu[1,2], Yashwant Kumar[3], Shakti Kumar[2], Sonu Kumar Gupta[3], Manas Ranjan Tripathy[1,2], Deepak Kumar Rathore[2], Amit Awasthi[1,2]*

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,

[1]Immunbiology Lab, Translational Health Science and Technology Institute, NCR-Biotech Science Cluster, 3rd Milestone, Faridabad-Gurgaon Expressway, Faridabad, Haryana 121001, India. [2]Infection and Immunology, Translational Health Science and Technology Institute, NCR-Biotech Science Cluster, 3rd Milestone, Faridabad-Gurgaon Expressway, Faridabad, Haryana 121001, India. [3]Noncommunicable Disease Center, Translational Health Science and Technology Institute, NCR-Biotech Science Cluster, 3rd Milestone, Faridabad-Gurgaon Expressway, Faridabad, Haryana 121001, India.
*Corresponding author. Email: aawasthi@thsti.res.in