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Entitled

CHARACTERIZATION OF IMMUNOMODULATORY PROPERTIES OF MANUKA HONEY: POTENTIAL MITIGATION AGAINST COLON CANCER

by

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Abstract

The focus on using alternative natural products for cancer prevention and treatment has been increasing over the past decade. Among these natural products, Manuka honey (MH) has been extensively investigated for its antimicrobial, wound healing, and anti-cancer effects. The current study aimed to characterize the immunomodulatory capacity of MH using both *in vitro* and *in vivo* approaches and investigate its potential to modulate anti-tumor immune responses in a preclinical model of colorectal cancer. We demonstrate that MH can directly act on macrophages to induce gene expression of several inflammatory cytokines and chemokines and the production of TNF-, a major pro-inflammatory cytokine. Moreover, intraperitoneal (i.p.) administration of MH into C57BL/6 mice elicited a peritoneal response characterized by a significant expansion in the number of peritoneal exudate cells (PECs), which was mainly due to a 35-fold increase in the recruitment of neutrophils. Importantly, this response was evident in toll-like receptor 4 (TLR4)-defective C3H/HeJ mice, indicating that the observed immunostimulatory effect occurs independently of TLR4 and, hence, is unlikely to be due to any lipopolysaccharide (LPS) contaminant. MH administration also led to changes in the phenotypic expression and functional maturation of peritoneal macrophages, as evidenced by a shift towards the $CD11b^{lo}$ $F4/80^{lo}$ phenotype, and an increase in the expression of major histocompatibility complex (MHC) class II proteins. In contrast, the peritoneal response was largely abrogated in mice deficient in the MyD88 protein, a critical adaptor of several pro-inflammatory signaling pathways. These findings were further extended by showing that oral administration of MH could induce type I/II IFN response and lead to enhanced expression of the IFN-inducible protein, stem cell antigen-1 (Sca-1), on lymphoid cells in peripheral lymphoid tissues, highlighting the ability of MH to trigger systemic immune responses. The importance of MH-induced immune responses was aptly demonstrated in a preclinical model of colorectal cancer. Using a preventative treatment approach, oral administration of MH led to a significant retardation in the growth of implanted tumors. We provide evidence that pretreatment with MH led to enhanced immunogenicity of tumors, as demonstrated by increased infiltration of immune cells into the tumor microenvironment, a ~2.0-fold increase in the percentages of intratumoral $CD4^+$ and $CD8^+$ T cells, and a 50% decrease in the percentage of $Ly6G^+$ granulocytic myeloid cells. Immunohistochemical staining of tumor tissues also showed enhanced infiltration by $CD4^+$ and $CD8^+$ T cells and an increase in the number of granzyme-B-expressing cells following MH treatment. Furthermore, intratumoral macrophages exhibited higher levels of MHC class II proteins, reflecting their critical role as antigen-presenting cells. Most importantly, our findings also demonstrate a significant increase in the percentage of tumor cells expressing high levels of MHC class I proteins in MH-pretreated mice, indicating an increase in tumor immunogenicity. Transcriptomic analysis of purified tumor-infiltrating leucocytes highlighted changes in the expression of various chemokines and inflammatory cytokines following MH treatment. Overall, the findings of the current study highlight the immunostimulatory properties of MH and demonstrate its potential utilization in cancer prevention.

Keywords: Manuka honey, Immunostimulatory response, Immunomodulatory agent, Colorectal cancer, Tumor microenvironment, Cancer prevention.