Modulation of the immune response by hydrogen sulfide in a model of colitis-associated cancer

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Chronic inflammation contributes to tumor initiation in colitis associated colorectal-cancer (CAC). Indeed, inflammatory bowel diseae (IBD) patients have an increased risk of developing colon cancer[1]. Chronic inflammation promotes tumor development through various mechanisms including the production of proangiogenic factors, matrix metalloproteinases, and damage-associated molecular pattern molecules, all of which drive myeloid-derived suppressor cells (MDSCs) accumulation and suppressive functions[2]. MDSCs are a heterogeneous population of immature myeloid cells that can be distinct in two subtypes: $CD11b^{+}Ly6G^{+}Ly6C^{low}$ (Gr-MDSCs) and $CD11b^{+}Ly6G^{-}Ly6C^{high}$ (Mo-MDSCs) that contribute to tumor growth and progression by suppressing T-cells and modulating innate immune responses[3]. Hydrogen sulfide (H_2S), an endogenous gaseous signaling molecule with anti-inflammatory properties, promote the resolution of colitis and enhance ulcer healing[4-5]. In the last few years it has also been studying its role in cancer development but it needs a better understanding. The aim of my project was to establish the role of H_2S during the pathogenesis of CAC and its effect on the immune cells involved. For this purpose we used an innate model of CAC in wich 129SvEvS6.*Rag2*$^{-/-}$mice were infected with *Helicobacter hepaticus (H.h.)*, a gram-negative, spiral-shaped, microaerophilic bacterium that is the main pathogen involved in IBD[6]. *H.h.* infection induces a significant and severe inflammation in the colon and in the cecum and also splenomegaly, already after 3 weeks. By flow cytometry we found that the progressive intestinal inflammation went along with a significant time-dependent increase of Gr-MDSCs and Mo-MDSCs in the colon and in the spleen, compared with uninfected mice. In particular, the Gr-MDSCs were higher than the Mo-MDSCs especially at 6 weeks. To understand the role of endogenous H_2S during colitis development we measured H_2S synthesis and the expression of H_2S synthesizing enzymes, cystathionine-beta-synthase (CBS) and cystathionine-gamma-lyase (CSE), in the colon of *H.h.-infected mice. The results demonstrated that* H_2S synthesis was significantly reduced in inflamed colon (**P<0.01) and this reduction was attributed to a down-regulation of both CBS protein and mRNA expression in the colon. To confirm the major role of CBS in colitis development we further inhibited CBS enzymatic activity in *H.h.-infected mice* by daily administration of a CBS competitive inhibitor, O-carboxymethyl-hydroxylamine hemihydrochloride (10mg/Kg), for 6 weeks. We found that CBS inhibition resulted in exacerbation of colitis (**P<0.01). Having established that endogenous H_2S synthesis in inflamed colon is markedly reduced we examined the effect of administration of exogenous H_2S donors on colitis development. Therefore, *H.h.*-infected mice were daily treated with L-cysteine (1g/Kg) or DATS (50mg/Kg) or vehicle (PBS) for two weeks. We found that only DATS led to a significant reduction of inflammation in the colon (*P<0.05). This anti-inflammatory effect was not due to eradication of the bacteria or decreased levels of colonization but was might linked to the ability of H_2S to reduce Gr-MDSCs subset in the colon and also to inhibit the pro-inflammatory activity of *H.h.* by reducing the production of TNFÎ± and IL6, as demonstrated by *in vitro* experiments on bone marrow derived macrophages (BMDMs). Taken together, these results suggest that H_2S may have a protective role in intestinal *H.h.-induced inflammation,* thanks to its ability to reduce the inflammatory response orchestrated by MDSCs that could trigger to colorectal cancer in presence of carcinogen agent.

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