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Dendritic Cells in Barrett's Esophagus Carcinogenesis

An Inadequate Microenvironment for Antitumor Immunity?

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Barrett's esophagus corresponds to the replacement of the normal esophageal squamous epithelium by a columnar epithelium through a metaplastic process. This tissue remodeling is associated with chronic gastroesophageal reflux and constitutes a premalignant lesion leading to a 30- to 60-fold increase in the risk to evolve into esophageal adenocarcinoma. The present study aimed to investigate a possible immune evasion in Barrett's esophagus favoring esophageal adenocarcinoma development. We demonstrated that myeloid and plasmacytoid dendritic cells are recruited during the esophageal metaplasia-dysplasia-carcinoma sequence, through the action of their chemoattractants, macrophage inflammatory protein 3α and chemerin. Next, we showed that, in contrast to plasmacytoid dendritic cells, myeloid dendritic cells, co-cultured with Barrett's esophagus and esophageal adenocarcinoma cell lines, display a tolerogenic phenotype. Accordingly, myeloid dendritic cells co-cultured with esophageal adenocarcinoma cell lines stimulated regulatory T cell differentiation from naïve $CD4^+$ T cells. In agreement with those results, we observed that both metaplastic areas and (pre)malignant lesions of the esophagus are infiltrated by regulatory T cells. In conclusion, soluble factors secreted by epithelial cells during the esophageal metaplasia-dysplasia-carcinoma sequence influence dendritic cell distribution and promote tumor progression by rendering them tolerogenic. *(Am J Pathol 2013, 182: 2168–2179; http://dx.doi.org/10.1016/j.ajpath.2013.02.036)*

Barrett's esophagus (BE) is defined by the replacement of the normal esophageal squamous epithelium by a metaplastic columnar epithelium containing true goblet cells on histological examination.[1] BE is considered as an adaptive response after chronic gastroesophageal reflux.[2] BE can be classified histologically into four groups [no dysplasia, indefinite for dysplasia, low-grade dysplasia (LGBE), or high-grade dysplasia (HGBE)], depending on the presence or absence of dysplastic cells in the epithelium. In the general population, BE prevalence is estimated to be between 1.6% and 6.8%.[2] This tissue remodeling is associated with a 30- to 60-fold increase in the risk of developing an esophageal adenocarcinoma (EAC),[2] and EAC almost always arises after a metaplasia-dysplasia-carcinoma (MDC) sequence. BE remains an important health challenge. First, survival outcomes for patients with EAC remain poor despite recent

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diagnostic and therapeutic improvements, with community 5-year survival rates of <20%.[3,4] Second, the incidence of BE has dramatically increased in the Western world during these past years, compared with other types of cancers, with an increment of >600%.[4,5]

Despite improved knowledge about the interactions between immunity and cancer, regulation of the immune system during the MDC sequence is not yet fully understood. BE and EAC constitute interesting models for chronic inflammation associated with a (pre)malignant disease.[4] For the most part, the development of BE and EAC is associated with a relative increase of type 2 helper T cells[6] and the presence of an immunosuppressive (IL-4, IL-6, and IL-10) cytokine pattern,[7] compared with gastroesophageal reflux–induced esophagitis characterized by a type 1 helper T-cell immune response, which is more appropriate for antitumor immunity.

Dendritic cells (DCs) are specialized antigen-presenting cells that provide a critical link between innate and adaptive immune responses. Human DCs are divided into two major intrinsically different subsets: myeloid DCs (mDCs), also called conventional DCs, and plasmacytoid DCs (pDCs), which differ from mDCs in their transcriptional program, phenotypic markers, and immunological functions.[8] mDCs play a crucial role in the regulation of adaptive immunity by their unique ability to induce a primary immune response in resting naïve T cells.[8,9] mDCs, characterized by the expression of the CD1a marker,[10,11] arise from bone marrow–derived myeloid progenitors and circulate in the peripheral bloodstream as precursors that home to tissues where they reside as immature cells with high endocytic activity and low T-cell activation potential.[12] These mDCs express CCR6 and are attracted via this chemokine receptor by macrophage inflammatory protein (MIP) 3α[13] in tissues. On exposure to danger signals [eg, lipopolysaccharide (LPS)], immature mDCs undergo maturation characterized by an up-regulation of costimulatory and antigen-presenting molecules.[9,14–17] *De novo* CCR7 expression allows these mature cells to migrate to local lymph nodes, where they can trigger an effective T-cell response.[17] Fully mature mDCs are characterized by the production of the IL-12 proinflammatory cytokine, which is required for the induction of an efficient T-cell response.[18] pDCs, the second subset of DCs, represent a rare population of human blood cells characterized by a rapid and massive secretion of type I interferon on activation via a Toll-like receptor–dependent recognition of pathogenic agents or danger signals. Immature pDCs present plasma cell–like morphological characteristics and selectively express CD4, CD45RA, CD123, BDCA-2, and BDCA-4, but are CD11c negative.[19] Under steady-state conditions, pDCs reside in secondary lymphoid tissues, from where they are mobilized to inflammatory sites[20] under pathological conditions, notably through the action of one of their chemoattractants, chemerin.[21] Through their capacity to conjugate innate and adaptive immunity and to secrete soluble factors controlling cancer development, these cells are crucial actors in antitumor immunity.[22]

mDC and pDC involvement in tumor immunity was shown to have a clinical impact because their infiltration in some primary tumor types has been associated with significant changes in patient survival and recurrent disease.[9,23–29] It has been postulated that tumors may evade the immune system by an impairment of DC number and functions mediated by a local production of immunosuppressive cytokines, such as prostaglandin E_2 (PGE_2), receptor activator of NF-κB ligand (RANKL), and IL-10, or by a modulation of chemokine expression.[30] Moreover, accumulating evidence suggests that mDCs and pDCs recruited to the tumor microenvironment often display tolerogenic properties by promoting regulatory T cell (Treg) expansion or proliferation.[31–33]

In this study, we tested the hypothesis that impaired antitumor immunity in BE could promote the development and progression of EAC. First, we evaluated the differential density of mDCs, pDCs, and Treg cells in the MDC sequence associated with BE. Then, we evaluated the expression of chemokines (MIP3α and chemerin) that could explain these changes of immune cell density in the MDC sequence. Finally, we performed *in vitro* studies to determine whether DC functionality is altered in the presence of soluble factors secreted by BE, HGBE, and EAC cell lines.

A better understanding of the factors involved in the transformation of BE into EAC through a deregulation of antitumor immunity could allow the development of new immunotherapeutic strategies leading to effective antitumoral responses.

Materials and Methods

Tissue Specimens

A total of 100 formalin-fixed, paraffin-embedded endoscopic biopsy or surgical specimens were obtained from the Tumor Bank of the University Hospital of Liege (Liege, Belgium). Samples were reviewed by two gastrointestinal pathologists (N.B. and J.S.) and included normal esophageal mucosa ($n = 20$), BE without dysplasia ($n = 20$), LGBE ($n = 20$), HGBE ($n = 20$), and EAC ($n = 20$). Only EACs arising in the esophagus in the presence of adjacent Barrett's metaplasia were considered. Each individual case was classified in accordance with the Vienna classification. We also analyzed the progression potential of these cases to more severe lesions in follow-up biopsy or surgical specimens. The protocol was approved by the Ethics Committee of the University Hospital of Liege, and all human subjects provided appropriate informed consent. Clinical and pathological data were available for each patient and are summarized in Table 1.

mDCs, pDCs, and Treg Cell Detection by IHC

Paraffin-embedded sections of esophageal biopsy specimens (4 μm thick) underwent immunostaining using an automated