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Tumor infiltrating lymphocytes in gastrointestinal tumors: Controversies and future clinical implications

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ABSTRACT

Chronic inflammation following infections, autoimmune diseases or exposure to environmental irritants plays a crucial role in tumor development and influences the host immune response to neoplastic cells. The presence of an anti-tumor immune infiltrate is often associated with better outcomes in gastro-intestinal primary cancers, particularly in those with high microsatellite instability (MSI-H). Immunotherapeutic drugs inhibiting the PD-1 and PD-L1 pathway showed promising results in the treatment of these patients in the metastatic setting. The aim of this review is to resume the role tumor infiltrating lymphocytes (TILs) play in gastrointestinal tumors, underlining their potential value as a prognostic and predictive biomarker. TILs assessment could identify subsets of patients with high extent of TILs and better prognosis, that could be spared from adjuvant systemic treatments. Immune infiltration parameters might be additional predictors of a greater benefit from the immunotherapy with the immune checkpoint blockade.

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1. Introduction

The interaction between neoplastic cells and the immune system starts during the early phases of tumor development, when strongly immunogenic cells are recognized and eliminated by the immune response. In the equilibrium and escape phases, the most aggressive and less immunogenic cells are able to survive and finally evade immunity, tumor becoming clinically manifested (Dunn et al., 2002). The presence of an extensive immune infiltrate has been associated with improved patients outcomes (better survival and responses to treatments) in a variety of solid tumors [ovarian, colorectal (CRC), non-small cell lung cancer (NSCLC), melanoma, HER2-positive and triple-negative (TN) breast cancer (BC), . . .] (Hwang et al., 2012; Galon et al., 2006; Zeng et al., 2016; Thomas et al., 2013; Salgado et al., 2015). More, the recent introduction of new immunotherapeutic agents, the immune checkpoint inhibitors, showed promising results in the treatment of several advanced neoplasms, like melanoma, kidney, NSCLC, bladder cancer and head and neck squamous cell carcinomas, giving rise to durable responses also in heavily pre-treated patients (Reck et al., 2016; Ferris et al., 2016; Larkin et al., 2015; Motzer et al., 2015; Borghaei et al., 2015; Rosenberg et al., 2016). In the adjuvant setting, higher rates of recurrence-free survival (RFS), overall survival (OS) and distant metastasis-free survival were observed in stage III melanoma patients treated with the anti-CTLA-4 ipilimumab (Eggermont et al., 2016). Nevertheless the activity of these drugs is limited to a proportion of subjects and is associated with non-negligible side effects, leading to a strong need for the identification of the best responders to these treatments.

1.1. The elements of the tumor immune environment: from innate to adaptive immunity

The tumor immune environment can be oriented towards an anti-tumor or alternatively to an inflammatory pro-tumor response, depending on the (dynamic) balance of the different subpopulations of its tumor infiltrating leukocytes and their functional status. For example, tumor associated neutrophils (TAN) and macrophages (TAM) can be polarized in different populations playing opposite roles, anti- (N1 and M1) and pro-tumor (N2 and M2) respectively (Gregory and Houghton, 2011; Kim et al., 2016). Myeloid derived suppressor cells (MDSC) are immature myeloid cells that dampen T cell activation in a not antigen (Ag) specific way, through different mechanisms (release of inhibitory molecules and deprivation of amino acids involved in the proliferation of lymphocytes) (Kumar et al., 2016). Dendritic cells (DC) are professional Ag presenting cells (APC) that migrate in and out of tissues and lymph-nodes; some DC subpopulations can be immune-suppressive, others are not (Volovitz et al., 2016). Natural killer (NK) cells target those cells that have lost the expression of MHC proteins (the so called missing-self) and can be also activated by stressed-induced ligands that are upregulated by tumor cells (namely the stress-induced self). Additionally, NK cells can shape the tumor microenvironment by producing cytokines that are involved in the differentiation, activation and recruitment of other immune cells (Vivier et al., 2012). In the context of adaptive immunity, T and B lymphocytes drive an Ag specific response, through T cell (TCR) and B cell receptors (BCR) respectively. T lymphocytes are differentiated in various subpopulations with distinct profiles: some subsets of CD4+ helper T cells can have anti- (Th1, Tfh, . . .), pro-tumor (i.e.: Th2) or regulatory functions (Treg); cytotoxic CD8+ T lymphocytes (CTL) mostly kill tumor cells and release cytokines (Chen et al., 2016). B lymphocytes can present Ags, produce antibodies and cytokines and also behave as regulatory cells (Breg), inhibiting the immune response (Siliņa et al., 2014; Gorosito Serrán et al., 2015) (Fig. 1).

Cytotoxic T lymphocytes (CTL) and natural killer (NK) cells exert a cytotoxic activity. Tumor associated macrophages (TAM) and tumor associated neutrophils (TAN) can play both anti-tumor or pro-tumor roles depending on their polarization (M1 and N1 or M2 and N2 respectively). Dendritic cells (DC) and B lymphocytes are antigen presenting cells (APC). B cells can also produce antibodies (Ab), secrete cytokines and behave as regulatory cells. T regulatory lymphocytes (Treg) secrete soluble factors (IL-10 and TGF-β, i.e.) and express high levels of the inhibitory immune checkpoint molecule CTLA-4 playing a suppressive role and regulating the activity of some other immune cells at the tumor site.

1.2. The assessment of the immune infiltrate in tumors

In order to investigate the clinical significance of the immune infiltrate in tumors, TILs evaluation has been done with various methods: gene signatures (analyzing the mRNA expression of specific cytokines and genes involved in the immune response, with activity and functional information) and pathological assessment on hematoxylin-eosin (H&E), immunohistochemistry (IHC) and immunofluorescence (IF) stained slides. H&E colored tumor sections are routinely used, and allow the evaluation of the extent of mononuclear inflammatory cells infiltration, which has a prognostic and predictive significance in TN and HER2-positive BC, i.e. The use of specific antibodies at IHC and IF (i.e.: anti-CD3, −CD20, −CD4, −CD8, markers for T, B, CD4+ and CD8+ T lymphocytes respectively) accurately identifies the various subpopulations of TILs (Salgado et al., 2015). Microscopic analysis gives information on TILs spatial distribution (at the invasive margin, in the stroma or within cancer cell nests) and density that have been shown to be prognostic in CRC (Galon et al., 2006).

1.3. TILs and benefit from immunotherapy

TILs have also been studied as predictive markers of response to the new immune checkpoint blockade (ICB) therapies. Metastatic melanoma patients who benefit more from the anti-PD-1 pembrolizumab had high extent of proliferating CD8+ TILs at the tumor invasive margin, PD-L1+, PD-1+ immune cells and a more clonal TILs TCR repertoire (Tumeh et al., 2014). The link between tumor genomics and better responses to immunotherapy has also been investigated in metastatic melanoma patients treated with the CTLA-4 blockade, where a high mutational load and the presence of specific neo-Ags predicted major clinical benefit from the treatment (Snyder et al., 2014). In NSCLC objective responses (OR), durable clinical benefit, and progression-free survival (PFS) improved during treatment with pembrolizumab. These responses were seen in the presence of an inflamed microenvironment together with activated effector T cells, in the presence of clonal neo-Ag and a high nonsynonymous mutation burden (McGranahan et al., 2016; Rizvi et al., 2015). Pembrolizumab administered in first line in advanced NSCLC patients with tumors expressing PD-L1 on at least 50% of neoplastic cells gave rise to longer PFS and overall survival (OS) with respect to standard platinum-based chemotherapy (Reck et al., 2016). In metastatic CRC patients the mismatch repair (MMR) deficiency status predicted benefit from the anti-PD-1 treatment. This condition, caused by the inactivation of MMR-associated genes following either epigenetic silencing or germline mutations (with an additional somatic inactivation of the second allele) generates a large number of somatic mutations (thousands *versus* hundreds that are found in mismatch-proficient tumors). These mutations accumulate in regions of repetitive DNA, leading to microsatellite instability (MSI). In patients treated with pembrolizumab, IHC expression of CD8 and PD-L1 by TILs was not significantly associated with the outcome(s) (Le et al., 2015).