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ORIGINAL PAPER

Immune Cells in Colorectal Cancer: Prognostic Relevance and Role of MSI

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Abstract There is growing evidence that both local and systemic inflammatory responses play an important role in the progression of a variety of solid tumors. Colorectal cancer (CRC) results from the cumulative effect of sequential genetic alterations, leading to the expression of tumor-associated antigens possibly inducing a cellular anti-tumor immune response. It is well recognized that cytotoxic lymphocytes (CTLs) constitute one of the most important effector mechanisms of anti-tumor-immunity. However, their potential prognostic influence in CRC remains controversial. In addition, other key players like natural killer cells, tumor associated macrophages and regulatory T cells play an important role in the immune attack against CRC and need further investigation. This review will mainly focus on the role of the adaptive immune system in CRC and particularly in regard to microsatellite instability.

Keywords Colorectal cancer · Immune response
T lymphocytes · Microsatellite instability

Introduction

Colorectal cancer (CRC) is a major public health problem. CRC results from the cumulative effects of sequential genetic alterations, leading to a progressive and irreversible loss of normal control of cell growth and differentiation. Several well-defined pathways are responsible for these transformations leading to genomic instability whereby widespread loss of DNA integrity is perpetuated [1]. In CRC, at least 3 distinct pathways of genomic instability have been described, the chromosomal instability (CIN), microsatellite instability (MSI), and CpG island methylator phenotype (CIMP) pathways. CIN is observed in 80%–85% of sporadic colorectal cancers; the term refers to an accelerated rate of gains or losses of whole or large portions of chromosomes that results in karyotypic variability from cell to cell. The consequence of CIN is an imbalance in chromosome number (aneuploidy), subchromosomal genomic amplifications, and a high frequency of loss of heterozygosity (LOH) [2].

Microsatellite instability (MSI) is the molecular fingerprint of a deficient DNA mismatch repair system observed in approximately 15% of CRC. Failure to repair replication-associated errors allows persistence of mismatch mutations all over the genome, but especially in regions of repetitive DNA known as microsatellites [3, 4]. Colorectal malignancies demonstrating MSI have a very heterogeneous histological appearance, improved prognosis and altered response to chemotherapy and radiotherapy [5, 6].

CIMP is a novel molecular instability pathway that appears to be responsible for most cases of aberrant tumor suppressor methylation in colorectal cancer, and which has important interactions with genetic pathways as well. In fact, CIMPtumors account for the majority of sporadic colorectal cancers with microsatellite instability, through methylation of the mismatch repair gene *hMLH1* [7].

At present, surgical removal of the primary tumor remains the mainstay of treatment for these solid tumors. The pathological assessment of the resection specimen describes the anatomic extent of the tumor (tumor-node-metastasis (TNM) categories). TNM staging estimates the

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postoperative outcome and rationale for adjuvant therapy. Despite the prognostic power of this staging system, determining the outcome for patients is still imprecise [8]. Genetic and molecular tumor prognostic factors have been proposed to identify patients who may be at risk for recurrence. None of these however, have been sufficiently informative for inclusion in clinical practice [9].

Accumulating evidence suggests that tumor progression is governed not only by genetic changes intrinsic to cancer cells but also by epigenetic and environmental factors [10]. Previous studies have suggested that immune infiltrates in CRC may be of clinical importance. To grow, invade and metastasize, a tumor interacts with its microenvironment composed of a diversity of cells of various origins that form the tumor matrix, its vascularization, its lymphatic and neurological network. The microenvironment also contains cells of the immune system including inflammatory infiltrates of the innate and the adaptive immune system [11]. All of them are scattered within the tumor and loaded with an assorted array of cytokines, chemokines and inflammatory and cytotoxic mediators. Cancer immunoediting is the ability of the immune system to control and shape cancer, and is the result of three phases, elimination-equilibrium-escape, that function either independently or in sequence [12]. While experimental evidence supports the idea that the innate immune system can promote tumor development through inflammation-dependent mechanisms, infiltration of the tumor by lymphocytes of the adaptive immune system may be a favorable prognostic sign [10]. However, the role of the adaptive immune reaction is still a matter of debate. The immunosurveillance theory has dominated the field for a long time but has been difficult to demonstrate. Although the presence of high numbers of tumor infiltrating lymphocytes is often a sign of good prognosis, there are many reports of local lymphatic inactivation, immune deviation, or the presence of regulatory T cells, which are associated with the occurrence of metastasis and poor clinical outcome [11]. In addition, tumor infiltrating T cells exist in an environment of chemokines and cytokines produced by the tumor cells, stromal cells as well as the infiltrating immune cells. The interactions between the malignant cells and the local immune infiltration are complex and result in a balance between tumor-promoting and tumor-controlling effects [11]. For clinicians, a greater understanding of the role of the host immune response in influencing the natural history of CRC might have important implications. It allows better risk stratification and could help guide recommendations regarding adjuvant therapy as well as the development of new immune based approaches to the treatment of CRC [13].

In this review we will mainly focus on the role of the adaptive immune system in CRC and particularly in regard to the microsatellite status.

Antitumor Effector Cells

Tumor Infiltrating Lymphocytes

Tumor infiltrating lymphocytes (TILs) are mainly T-cells characterized by the presence of the cluster of differentiation 3 (CD3) surface protein. They infiltrate many different tumor types and are believed to be one of the most important specific antitumor effector cells. Two subgroups of T cells can be identified within the TIL population, respectively: $CD8^+$ (cytotoxic) and $CD4^+$ (helper) T cells [14].

The Adaptive Immune System and Cancer

$CD8^+$ T cells are referred to as cytotoxic T cells (CTLs) given their ability to kill "target cells". In order for $CD8^+$ T cells to recognize antigens, these need to be exposed on the tumor cells in association with the human leukocyte antigen (HLA) class I proteins. Upon encounter of a tumor cell antigen/HLA I complex for which their αβ-T-cell receptor is specific, $CD8^+$ cells clonally expand and subsequently differentiate into killer cells. Part of the differentiation process includes the formation of a large number of modified lysosomes loaded with perforin and several types of granzymes, and, on the other hand, increased expression of the death-activator-designated Fas-Ligand (FasL) [14, 15]. These differentiated CTLs can mediate specific destruction of tumor cells by the release of their lytic components in case of direct cell-cell interaction. Perforins cause disruption of the cell membrane by creating pores that enable the enzymatic proteases (such as granzyme B) to enter the cell, where they cleave precursors of caspases inducing autodestruction of the tumor cell or apoptosis. If the tumor cell expresses the death receptor, Fas, Fas-L/Fas interaction is believed to be of major importance [14, 16, 17]. While most CTLs die through apoptosis following effectuation of their killer function, some become long-lived memory cells [14].

In addition, other factors of the adaptive immune system play a role in the cancer immunosurveillance. $CD4^+$ T cells, which only respond to antigens presented by the HLA class II proteins expressed by antigen presenting cells (like dendritic cells, macrophages and B-cells), are important for antitumor immunity. $CD4^+$ T cells autoregulate their proliferation by releasing interleukin 2 (IL-2) that binds to the IL-2 receptor on the cell surface of the $CD4^+$ T cell. After many cell generations, the progenitor cells differentiate into memory, suppressor and effector cells [14]. Depending on the cytokine profile produced by the effector cells, $CD4^+$ T cells are subdivided in T helper 1 (Th1) cells or T helper 2 (Th2) cells, each secreting specific cytokines. Once an immune response is triggered to generate Th1

cells, the production of Th2 cells is actively suppressed by the Th1 cytokines and vice versa. Importantly, Th1 cells are essential for the proliferation of CTLs as CTLs require Th1 effector cell produced IL-2 for their proliferation. Although it has been shown that $CD4^+$ T cells may be sufficient by themselves to eliminate tumor cells, it is more often the case that both $CD4^+$ and $CD8^+$ T cells are required for an effective tumor cell elimination because most tumor cells only express HLA class I molecules [14].

Natural Killer Cells

The induction of CTL responses entails a certain delay, leaving time for the tumor cells to escape the immune system. Therefore, natural killer (NK) cells from the innate immune system may also play an important role since these cells can lyse NK-sensitive tumor targets prior to antigen sensitization or clonal expansion. Given that NK cells are not HLA restricted, these cells bear the capacity to eliminate tumor cells that do not express the HLA complex. In contrast, HLA complexes are recognized by inhibitory receptors on NK cells resulting in their inactivation. In addition, NK cells express several ligands of the tumor necrosis factor family and can induce apoptosis of malignant cell targets, which are phagocytosed by dendritic cells and macrophages and processed for subsequent presentation to T cells. Furthermore, NK cells constitutively express the IL-2 receptor and are able to respond to IL-2 stimulation that results in augmented cytotoxic activity [14]. Owing to these cytotoxic capacities, it was not surprising to learn that a decreased number of preoperative NK-cells in patients with CRC was associated with an increased frequency of postoperative tumor recurrence [18].

Tumor Associated Macrophages

Tumor-associated macrophages (TAMs), which constitute a significant part of the tumor-infiltrating immune cells, have been linked to the growth, angiogenesis, and metastasis of variety of cancers, including breast and cervical cancers and transitional cell carcinomas [19], most likely through polarization of TAMs to the M2 (alternative) phenotype. M2 macrophages are diverse, but in general are involved in T helper 2 (Th2) response, have an immunoregulatory function, and orchestrate encapsulation and containment of parasites and promote tissue repair, remodeling, and tumor progression. In contrast, M1 (classical) macrophages are generally characterized by interleukin (IL)–12^{high}, IL-23^{high}, IL-10^{low} phenotype. They produce reactive oxygen and nitrogen intermediates as well as inflammatory cytokines and play a role in Th1 responses. Finally, M1 macrophages mediate resistance against intracellular parasites and tumors.

The role of TAMs in colon cancer is more controversial, as most articles indicate that peritumoral TAMs prevent tumor development (suggesting polarization of TAMs towards the M1 (classical) phenotype); patients with high TAMs numbers have better prognosis and survival rate [20–23]. In contrast, intratumoral TAMs count has been correlated with depth of invasion, lymph node metastasis, and staging of CRC, suggesting that intratumoral macrophages cause cancer cells to have a more aggressive behavior [24, 25].

In case of M2 polarization, several mechanisms for a worse prognosis have been proposed. A positive feedback loop has been identified between tumor cells and macrophages that propagates the growth and promotes the survival of colon cancer cells: tumor cells stimulate macrophages to secrete IL-1β, which in turn, promotes Wnt signaling and stabilizes Snail in tumor cells, conferring resistance to TRAIL [26]. Furthermore, IL-1β and TAMs activate NF-κB-dependent PDK1/AKT signaling in tumor cells, and thereby inactivate GSK3β, enhance Wnt signaling and promote growth of colon cancer cells, suggesting another molecular link between inflammation and tumor growth [27]. Macrophages exposed to hypoxia accumulate both HIF-1α and HIF-2α, and overexpression of HIF-2α in TAMs is specifically correlated with high-grade human tumors and poor prognosis. HIF-2α directly regulates proinflammatory cytokine/chemokine expression in macrophages activated in vitro [28]. This is of importance since in the majority of solid tumors, including bladder, brain, breast, colon, ovarian, pancreatic, prostate, and renal carcinomas, nuclear expression of HIF-2α was observed in subsets of the tumor cells, and was also strongly expressed by subsets of tumor-associated macrophages. In contrast, in normal tissue no HIF-2α was detectable except within bone marrow macrophages, where it was strongly expressed [29]. On the other hand, microarray analysis and functional testing revealed CSF-1 as the major chemoattractant for macrophages. In the chick *chorioallantoic membrane* model of cancer progression, macrophages are localized specifically to the tumor periphery where they were found to increase tumor growth, microvascular density, vascular disruption, and lung metastasis, suggesting these cells home to actively invading areas of the tumor, but not the hypoxic core of the tumor mass. This was further supported by the finding that hypoxic conditions downregulated CSF-1 production in several tumor cell lines and decreased macrophage migration in vitro [30]. Confusingly, activated macrophage-conditioned medium (AMCM), containing the M1 associated cytokines TNF-α, IL-1β, and IL-6, markedly induced proliferation and migration of human colon cancer cells HCT116. Furthermore, activated macrophage-conditioned medium significantly increased activation of transcription factor NF-κB and secretion of vascular endothelial growth factor (VEGF)

from colon cancer cells, which subsequently induced capillary morphogenesis of human aortic endothelial cells [31]. This shows that the interaction between tumor cells and TAMs is very complex and suggests a large grey area between the extremes M1 and M2.

Immune Escape Mechanisms

Despite the fact that malignant cells express specific antigens, thereby triggering a cytotoxic T cell response, and the NK cells also have distinct killing abilities, the immunosurveillance system apparently fails in the consequent detection and removal of malignant cells as the result of escape strategies established during development [14]. The escape mechanisms may result from altering or losing the HLA/peptide expression or through antigen modulation as well as from active biosynthesis of immunosuppressive molecules. These factors include immunosuppressive cytokines (e.g. IL-10, IL-8, TGF-β), prostaglandins, and VEGF, which redirect immune responses toward a favorable environment for growth [12, 14, 32]. Additionally, regulatory T cells (Treg) contribute to an immunosuppressive microenvironment through a cyclooxygenase-2-prostaglandin-2-dependent mechanism, direct cell-cell contact, or by the release of cytokines, like TGF-β, thereby facilitating tumor growth (see further below) [1, 33, 34]. Furthermore, TAMs, especially from the M2 phenotype, also release factors that favor growth and metastasis (as described above). Finally, malignant cells may actively eliminate immune effector cells through activation induced cell death or through the Fas counterattack [14].

Biology of Infiltrating T Cells in CRC

CRC become clinically malignant after cancer cells invade through the muscularis mucosa into the submocosa. With the invasion, various immune/inflammatory responses by competent immune effector cells, critically involved in the protection of the host organism against cancer, take place [1]. These responses are more concentrated along the invasive margin [13, 16]. Tumor-associated antigens (TAAs), expressed as a consequence of genetic alterations, are exposed on the tumor cells in association with HLA class I proteins and can be recognized by CTLs via the T cell receptor, finally resulting in a cellular immune response effective in limiting tumor growth and spread [16, 35]. Immune cells are present in the tumor stroma at the periphery of the tumor and occasionally invade cancer cell nests. Koch et al. [36] were the first to show a functional reactivity of tumor infiltrating T cells against antigens in CRC patients. In addition, they demonstrated for the first time tumor selective activation and cytotoxic activity in situ of tumor infiltrating $CD8^+$ T cells and tumor selective migration of $CD4^+$ T helper cells in colorectal cancer.

The potential influence of these immune-cell infiltrates in CRC on the prognosis of patients is investigated in several studies but remains controversial. A greater understanding of the role of the host immune response in influencing the natural history of CRC might have important implications for risk stratification and the development of adjuvant immune-based therapies [13, 37].

Tumor-Infiltrating Lymphocytes and Survival

The pioneering studies of Svennevig et al. [38] and Jass et al. [39] showed improved prognosis in CRC patients when a prominent lymphocytic infiltrate was present. Since then, the correlation between survival and number of TILs has been addressed in several studies [13]. Ropponen et al. [40] confirmed the concept of a prognostic impact of peritumoral and stromalTILs in CRC. In addition, they also showed an inverse correlation between the presence of TILs and tumor stage, i.e. TILs are more present in early stages (stage I and II) and decrease in advanced stages (stage III and IV). Several subsequent studies also demonstrated that the infiltration of TILs, in particular $CD8^+$ T cells, within and around tumor stroma contribute to a better prognosis (Table 1). In addition, some reports found that immune responses taking place in regional lymph nodes, as well as the number of circulating lymphocytes, in particular T cells and NK cells, may be associated with a better clinical outcome [21, 41–43].

The recent and methodologically extensive publications by Galon et al. [8, 44] and Pagès et al. [10, 11, 45] were able to dramatically augment the current knowledge regarding the role of T cell mediated immune responses in controlling the growth, progression and prognosis of CRC. While the early study of Pagès et al. [10] concentrated on the role of tumor-infiltrating immune cells in the early metastatic invasion of CRC, Galon et al. [44] dealt with the capacity of the adaptive immune response to control the behavior of CRC. They demonstrated that an increased intratumoral expression of markers for cytotoxic T cells was significantly associated with the absence of early metastatic events and with a decreased rate of cancer recurrence. It was clear that an effective immunological control of tumor metastasis and tumor progression is strongly driven by Th1-polarized cytotoxic effector T cells, as well as by the long-lasting antitumor capacity of $CD45R0^+$ memory T cells [10]. In addition, Galon et al. [44] showed that the type, density and location of immune cells in CRC had a prognostic value that was superior to TNM classification. The assumption that not only the number of tumor-infiltrating $CD8^+$ T lymphocytes, but also their cytolytic

Table 1 Role of TILs in prognosis of colorectal tumors. (BM-like: basal membrane like, CCR5: chemokine (C-C motif) receptor 5, CD: cluster of differentiation, CRC: colorectal cancer, CTL: cytotoxic T lymphocytes, FACS: fluorescence-activated cell sorting, FOXP3: forkhead box P3, HLA: human leukocyte antigen, IFN-gamma ELISPOT assay: interferon gamma enzyme-linked immunosorbent spot assay, IHC: immunohistochemistry, IM: invasive margin, it: intratumoral, MSI: microsatellite instability, NK cells: natural killer cells, RT-PCR: real time polymerase chain reaction, TCR: tumor cell receptor, TGF-β: transforming growth factor beta, TILs: tumor infiltrating lymphocytes, TMA: tissue micro-array)

Author	Investigated cell type	Methods	Conclusion
Ropponen et al., 1997 [40]	TILs	IHC	TILs can provide important prognostic information in CRC to be used in evaluating for adjuvant therapy in different tumor stages.
Naito et al., 1998 [48]	CD8, Granzyme B	IHC	Human colorectal cancer tissue was infiltrated by various numbers of T cells that had cytotoxic phenotype, contributing to a better survival of patients. This infiltration of colorectal cancer cell nests by $CD8^+$ T cells could be a novel prognostic factor.
Linnebacher et al., 2001 [49]	CD40 ligand B-cells, HLA-A2.1, CD3 and CD8 T cells	Peptide pulsed autologous CD40-activated B cells to generate CTL recognizing HLA-A2.1-restricted peptides, ELISPOT, chromium release and immu nofluorescence assay	Frameshift protein sequences represent a novel subclass of tumor-specific antigens. Therefore, it is tempting to speculate that a framshift peptide-directed vaccination approach could not only offer new treatment modalities for existing MSI tumors but might also benefit asymptomatic at-risk individuals in HNPCC families by a prophylactic vaccination strategy.
Oberg et al., 2002 [21]	CD8, CD45R0 and CD68	IHC	The presence of $CD8^+$, $CD45R0^+$ and $CD68^+$ immune cells in regional lymph node metastases may serve as predictors of patients survival in CRC Dukes' stage C.
Funada et al., 2003 [22]	CD8, CD68	IHC	Both $CD8^+$ T cell and macrophage peritumoral infiltration indicates anti-tumoral action in patients with colorectal cancer.
Diederichsen et al., 2003 [50]	CD4, CD8 and HLA-DR	Flow cytometry	Patients with a low $CD4^+/CD8^+$ ratio had a better clinical outcome, CRC cells might be a target for cytotoxic T-lymphocytes, however, the tumor cells are not able to initiate an immune response.
Menon et al., 2004 [51]	CD4, CD8, CD56, CD57, HLA-A/B/C, BM-like structure and MSI	IHC	The infiltration of $CD8^+$ and $CD57^+$ cells are important prognostic factors in CRC. However, their interaction with tumor cells is inversely correlated to the presence of HLA-I on tumor cells and a thick BM-like structure around tumor islets. Loss of hMLH1 expression was also correlated with a significantly higher intraepithelial $CD8^+$ and $CD57^+$ cell infiltration. In addition, NK cells might play an important role in the immune surveillance in CRC patients.
Chiba et al., 2004 [52]	Intraepithelial CD8 T cells	IHC	The effect of intraepithelial $CD8^+$ T cells may be mediated by suppression of micrometastasis, rather than the suppression of growth in the primary tumor.
Milasiene et al., 2005 [42]	CD3, CD8, CD4, CD20 and CD16	Immunoflourescence	The number of circulating lymphocytes and in particular T cells and NK cells may be associated with a longer survival of CRC patients in stage III and IV.
Pages et al., 2005 [10]	Local immune response	Flow cytometry, low-density-array RT-PCR and TMA	Signs of an immune response within CRC are associated with the absence of pathological evidence of early metastatic invasion and with prolonged survival.
Koch et al., 2006 [36]	CD8, CD4, CD69, CD107a	FACS, IFN-gamma ELISPOT analysis	Tumor-selective activation and cytotoxic activity of $CD8^+$ TIL and tumor-selective migration of $CD4^+$ T helper cells were demonstrated in CRC for the first time. These data support the immunogenicity of CRC and suggest clinical significance of tumor-specific immune responses.
Galon et al., 2006 [44]	Tumor infiltrating immune cells	Gene expression profiling and in situ IHC	The immunological data were found to be a better predictor of patient survival than the histopathological methods currently used to stage CRC. The data support the hypothesis that the adaptive immune response influences the behavior of human tumors.

Table 1 (continued)

Author	Investigated cell type	Methods	Conclusion
Baker et al., 2006 [53]	CD8 and TGF-β	IHC	Refractoriness to normal TGF-β signaling in CRC plays a role in the retention of lymphocytes within tumor epithelium.
Galon et al., 2007 [8]			Discussion of the meaning and potential implication of the finding that the type, density and location of immune cells in CRC could provide a prognostic factor superior and independent to that of criteria related to the anatomic extent of the tumor.
Pages et al., 2008 [11]			The nature, functional orientation, density, and location of immune cells within distinct tumor regions could provide a prognostic factor superior to and independent of criteria related to the anatomic extent of the tumor.
Wagner et al., 2008 [54]	CD3, CD8, CD4, CD25, CD69, CD107a	Multicolor flow cytometry and interferon gamma ELISPOT analysis	$CD4^+$ and $CD8^+$TILs are selectively activated in liver metastases, and cytotoxic T lymphocytes exert tumor-selective cytotoxic activity in situ in the presence of activated T helper cells, suggesting the requirement of in-situ-activated T helper cells for efficient cytotoxic T lymphocytes effector function.
Morris et al., 2008 [55]	TIL		Patients with TILs or perforation seemed to gain more survival benefit from chemotherapy. These results suggest there might be interactions between the immune system and chemotherapy leading to improved survival of colon cancer patients.
Qiu et al., 2009 [43]	CD3, CD4 and CD8 T cells and NKC	Flow cytometry	Measurement of cellular immunity in the peripheral blood of patients with CRC identified associations between immune status and clinical outcome.
Lugli et al., 2009 [56]	CD8 and CK22 double staining	TMA	The $CD8^+$ T/tumor budding index is an independent prognostic factor in CRC and a promising approach for a future prognostic score for patients with this disease.
Laghi et al., 2009 [47]	CD3	Computer assisted image analysis	The density of $CD3^+$ TIL (IM) cannot be used as an independent predictor of clinical outcome in patients with stage III CRC, and at least for now, the TNM classification should remain the preferred prognostic system.
Ogino et al., 2009 [57]	Four components of lymphocytic reaction (Crohn's like reaction, peritumoral reaction, intratumoral reaction and tumor infiltrating lymphocytes)	IHC, pyrosequencing and qRT-PCR	Lymphocytic reactions to tumor were associated with improved prognosis among CRC patients, independent of lymph node count and other clinical, pathological and molecular characteristics.
Mlecnik et al., 2009 [58]	Chemokines and adhesion molecules	Data integration and biomolecular network reconstruction	The expression of specific chemokines and adhesion molecules were found as being critical for high densities of T cell subsets within the tumor, and associated with particular TCR repertoire. Intratumoral-specific TCR use correlated with the prognosis of patients.
Pages et al., 2009 [45]	CD8, CD45R0	Large scale RT-PCR and TMA	The combined analysis of $CD8^+$ and $CD45R0^+$ cells in specific tumor regions could provide a useful criterion for the prediction of tumor recurrence and survival in patients with early stage CRC.
Roxburgh et al., 2009 [59]		Jass and Klintrup's criteria	A higher grade of local inflammatory response may represent effective host immune response impeding tumor growth.
Roxburgh et al., 2009 [60]		Jass and Klintrup's criteria and Glasgow prognostic score	Low peritumoral infiltrate (Klintrup criteria) and increased systemic inflammation (mGPS criteria) are linked through the cell-mediated immune system.

Table 1 (continued)

Author	Investigated cell type	Methods	Conclusion
			Furthermore, both pathological (Klintrup) and bio chemical (mGPS) measures of the inflammatory response predict survival after CRC surgery.
Suzuki et al., 2010 [61]	CD8, FOXP3	IHC	itCD8$^+$T/itFOXP3$^+$ cell ratio is a predictive marker for both disease free time and overall survival time in patients with CRC. Tumor producing TGF-beta may contribute to the increased number of FOXP3$^+$ cells.
Zimmerman et al., 2010 [62]	CCR5	RT-PCR	Intermediate and strong CCR5 expression was significantly associated with nonmetastatic CRC and increased CD8$^+$ T cell infiltration.

capacity determines the effectiveness of the immune system mediated tumor control was further supported by Atreya et al. [46]. They showed that the expression of the T-box transcription factor eomesodermin, which is critically involved in controlling cytolytic activity of CD8+ T cells, inversely correlates to the occurrence of lymph node metastasis in CRC patients [46]. In contrast, Laghi et al. [47] found that the density of CD3$^+$ cells in the invasive margin of CRC could not be used as an independent predictor of survival, and at least for now, the TNM classification should remain the preferred prognostic system.

Tumor-Infiltrating Lymphocytes and MSI-Status

Predominantly consisting of healthy 'self' and lacking of costimulatory and danger signals, tumor cells are generally considered as poorly immunogenic and, by failing to activate antigen presenting cells, may 'passively' anergize effector T cells [63]. In contrast, pronounced lymphocytic infiltration is marked in high-graded microsatellite instable (MSI-H) CRC and might explain the better clinical outcome in these patients [64]. It has been postulated that MSI-H CRC are more immunogenic than microsatellite stable (MSS) tumors because of the generation of a large number of abnormal peptides by frameshift mutations [49, 65–70]. However, in the analysis of Chiba et al. [52] there was no significant increase in the number of CD8$^+$ IEL in cases with MSI. Therefore, it appears that although MSI could be associated with a high infiltration of T cells in tumor tissue, additional mechanisms like cross-priming of antigen-presenting cells by released intracellular antigens or the use of HLA class II machinery and T helper cell activity may provide an alternative pathway for immune stimulation in vivo [41, 65, 67].

Role in Prognosis

For a better prognostic assessment, Dolcetti et al. [71] suggested the combination of evaluation of local lymphocytosis and the MSI status. MSI in combination with a high content of intraepithelial lymphocytes was found to be related to an improved overall survival in a group of exclusively right-sided CRCs. Consequently it has been suggested that these lymphocytes may actually represent an immune response that contributes to improved survival of MSI-H CRC and subsequent work has confirmed a possible link (Table 2). Guidoboni et al. [72] showed a significantly higher prevalence of activated CD8$^+$ IEL in MSI+ versus MSI- CRC, suggesting that the occurrence of a local cytotoxic immune response may be the major determinant of the good clinical course of these MSI+ CRC patients. Dolcetti et al. [71] and Michael-Robinson et al. [73] demonstrated that the increased frequency of TILs associated with MSI-H cancers are only weakly or moderately correlated with tumor apoptosis. However, while TILs might be expected to explain the increased apoptotic rate and improved prognosis of MSI-H cancers, it is likely that MSI-H cancers are intrinsically more prone to apoptosis, independently of T cell attack [73]. In addition, Buckowitz et al. [74] and Chiba et al. [52] suggested a protective role of functionally active lymphocytes directed against MSI-H CRCs, which may prevent tumor cell dissemination and metastasis formation in distant organs. Furthermore, it has been postulated that the abundance of CD8$^+$TILs found in MSI-H CRC might be due to the failure of these tumor cells to upregulateFasL. The failure to eliminate these CTLs by FasL induced apoptosis may explain, in part, the improved prognosis associated with MSI-H tumors [75]. Finally, an alternative approach reflecting the dynamic at the CRC tumor front has been studied using an index including tumor budding and CD8$^+$ lymphocytes. A tumor bud is typically defined as a single tumor cell or tumor cell cluster up to five cells at the invasive front. Tumor budding has previously been found to be associated with higher N stage, higher tumor grade and presence of vascular invasion, local tumor recurrence, distant metastasis as well as worse survival [76, 77]. The results of Lugli et al. showed that the CD8$^+$ lymphocyte to tumor budding index is an

Table 2 Role of TILs on prognosis of MSI colorectal tumors. (APM: antigen processing machinery, CD: cluster of differentiation, CLR: Crohn's like reaction, CRC: colorectal cancer, CTL: cytotoxic T lymphocyte, ELISPOT: enzyme-linked immunosorbent spot assay, FASL: Fas-ligand, HLA: human leukocyte antigen, IgG ab: immunoglobuline G antibody, IHC: immunohistochemistry, INF-gamma: interferon gamma, IL-2: interleukin-2, IL-8: interleukin-8, IUCC: international union against cancer, LI: lymphocyte infiltration, MSI: microsatellite instability, MSI-L: low grade of MSI, MSS: microsatellite stability, RT-PCR: real time polymerase chain reaction, SEREX: serological analysis of recombinant cDNA expression libraries, TILs: tumor infiltrating lymphocytes, Treg: T regulatory cell, TUNEL: Terminal deoxynucleotidyl transferase dUTP nick end labeling)

Author	Investigated cell type	Methods	Conclusions
Dolcetti et al., 1999 [71]	CD3, CD8, TIA1, Granzyme B and apoptotic cell death	IHC and TUNEL assay	MSI was the major determinant of the presence of activated cytotoxic intraepithelial lymphocytes. Moreover, MSI CRC also showed a significantly higher percentage of tumor cells undergoing apoptotic cell death, often located in close proximity of activated cytotoxic lymphocytes.
Guidoboni et al., 2001 [72]	CD3, CD8, Granzyme B	IHC	The presence of local cytotoxic immune response is probably the major determinant of the good clinical course of patients with MSI colon cancer. In addition, these patients may identify a subset of colon cancer patients with a favorable clinical outcome, particular in stage III disease.
Linnebacher et al., 2001 [49]	CD40 ligand B-cells, HLA-A2.1, CD3 and CD8 T cells	Peptide pulsed autologous CD40-activated B cells to generate CTL recognizing HLA-A2.1-restricted peptides, ELISPOT, chromium release and immunofluorescence assay	Frameshift protein sequences represent a novel subclass of tumor-specific antigens. Therefore, it is tempting to speculate that a framshift peptide-directed vaccination approach not only could offer new treatment modalities for existing MSI tumors but also might benefit asymptomatic at-risk individuals in HNPCC families by a prophylactic vaccination strategy.
Michael Robinson et al., 2001 [73]	CD3, CD8, CD20 and apoptosis	IHC	While TILs might be expected to explain the increased apoptotic rate and improved prognosis of MSI-H cancers, it is likely that TILs and apoptosis are independent characteristics of MSI-H cancers.
Menon et al., 2002 [87]	HLA-A/B/C	IHC	HLA class I was down regulated in 72% of CRC and provide independent prognostic information for a longer disease free survival. The better prognosis may be caused by elimination of HLA-negative cells by NK cells or by an attenuated tumor aggressiveness, as seen in tumors with MSI.
Lovig et al., 2002 [88]	HLA-DR	IHC	HLA-DR expression is correlated with a better survival in MSI-H, but also MSI-L and MSS CRC patients. This might be explained by enhanced T-cell mediated anti-tumor immune responses against tumor cells in the HLA-DR positive tumors.
Ishikawa et al., 2003 [68]	Tumor antigen and specific IgG ab	SEREX	Tumor specific peptides generated by MSI may be involved in antitumor immune response and may be useful for the development of diagnostic and therapeutic methods for patients with MSI CRC.
Prall et al., 2004 [80]	CD8 and MSI	Immunostaining	CD8 tumor infiltrating lymphocytes were identified as a promising candidate for further evaluation in the ongoing search for prognostic and predictive factors of CRC, particularly if combined with MSI status.
Takemoto et al., 2004 [89]	CD3, CD4, CD8, S100	IHC	This study suggests that different factors are involved in stroma-infiltrating lymphocytes (SIL) and intra-tumor cell-infiltrating lymphocytes (ITCIL). The cumulative survival rates tended to be higher in severe ITCIL. They suggest that there might be a possibility of ITCIL having a role for a better prognosis after CRC surgery, which is closely related to MSI.
Phillips et al., 2004 [65]	T cell markers, CD3, CD8, Granzyme B and IL2Rα	Quantitative fluorescent hydrolysis probe-based RT-PCR and IHC	MSI-H tumors contained higher ratios of $CD3^+/CD8^+$ mRNA copy numbers and more infiltrating lymphocytes. MSI-H CRC may be more immunogenic than MSS tumors.
Kloor et al., 2005 [90]	APM components, HLA class I, β2m,	IHC and mutation analysis	Defects on HLA class I antigen processing and presentation seem to be significantly more frequent in MSI-H than in

Table 2 (continued)

Author	Investigated cell type	Methods	Conclusions
			MSS CRC, suggesting that in MSI-H CRC the immune selective pressure leads to the outgrowth of cells with defects of antigen presentation.
Buckowitz et al., 2005 [74]	CLR and MSI	IHC and PCR	The MSI-H phenotype and the presence of CLR were independent predictors of a low UICC stage. These data, together with the recent definition of highly immunogenic neo-antigens expressed in MSI-H tumor cells, suggests that MSI-H CRC elicit a protective host response that may prevent metastasis formation.
Baker et al., 2007 [78]	CD8	TMA	TIL infiltration was found to correlate with multiple clinico-pathological features but was a prognostic marker only in MMR proficient CRCs
Dierssen et al., 2007 [91]	HLA class I, β2microglobulin and APM	IHC	Sporadic and hereditary MSI-H tumors follow different routes towards HLA class I loss of expression supporting the idea that these tumors follow different evolutionary pathways in tumorigenesis. The resulting variation in immune escape mechanisms may have repercussions in tumor progression and behavior.
Schwitalle et al., 2008 [69]	Tumor infiltrating T cells	ELISPOT assay and in vitro killing assays	Frameshift specific peptides presented by DNA mismatch repair deficient CRC cells are effectively recognized by the patient's immune system and may explain the characteristic clinicopathologic features of HNPCC-associated but also sporadic MSI-H CRCs.
Houston et al., 2008 [75]	CD3, FasL	IHC	The abundance of TILs found in MSI-H tumors may be due to the failure of these tumor cells to up-regulate FasL and may explain, in part, the improved prognosis associated with these tumors.
Michel et al., 2008 [92]	Treg	IHC	The elevated number of CD8-positive lymphocytes found in MSI-H CRC is paralleled by an enhanced infiltration with CD8-negative FOXP3-postive cells suggesting that FOXP3-positive cells may play a role in the regulation of the immune response directed against MSI-H CRC at the primary tumor site.
Tougeron et al., 2009 [70]	CD3 and frameshift mutations	Fluorescent multiplex PCR comparative analysis and IHC	Tumor infiltrating lymphocyte density was associated with the overall number of frameshift mutations within two target genes (ASTE/HT001 and PTEN). These results strongly argue for the clinical relevance of immunotherapy of CRC with MSI.
Banerjea et al., 2009 [93]	CD8, INF-gamma and IL-8	Bromodeoxy-uridine incorporation assay and ELISA	MSI-H cancers display enhanced immunogenic properties but the immune response to microsatellite and chromosome stable cancers appears to be absent and this may contribute to the poor prognosis of the latter two tumor types.
Drescher et al., 2009 [94]	CD3, CD8, CD25	IHC	The survival advantage enjoyed by patients with MSI-H CRC may, in part, be attributed to the increased cytolytic response, but not on an antigen-specific immunosuppressive response in MSS patients.
Chang et al., 2009 [95]	MSI and LI	IHC	Patients with colon cancer exhibiting both MSI^+ and LI^+ tumors have more favorable disease free survival rates. Both MSI and LI show promise as a combined prognostic marker and with further study may prove to be particularly useful in selecting patients with stage II disease for adjunctive therapy.
Deschoolmeester et al., 2010 [79]	MSI, CD4, CD8, Granzyme B	IHC	A role for infiltrating $CD3^+$ and $CD8^+$ T lymphocytes in colorectal cancer is suggested whereby tumor infiltration could reflect a general principle of antitumor immunity, irrespective of the MSI-status.

independent prognostic factor in CRC and represents biologically a pro-/anti-tumor model that could be a promising approach for a future prognostic score in CRC [56].

Nevertheless, several studies analyzing CRC samples stratified by MSI-status also report a positive effect of $CD8^+$ T cells in mismatch repair proficient cells [78, 79]. They also pointed out that perturbations in the TGF-β signaling pathway play an important role in the recruitment and retention of TILs within CRC epithelium [78]. Although Prall et al. [80] also found a prognostic impact of high $CD8^+$ densities in MSI-H CRCs, they showed that it was not solely restricted to this group. Therefore, they hypothesized that tumor infiltration by $CD8^+$ lymphocytes could reflect a general principle of antitumor immunity, irrespective of the MSI status [80].

With regard to the explicit prognostic relevance of these CTLs and other components of the immune system in the setting of CRC, therapeutic tools that are able to influence these immunological key mediators present promising candidates for more successful clinical control of progression, metastasis and recurrence of CRC. These new therapeutic approaches have already been successfully tested in various animal models of CRC or even in first clinical trials, demonstrating an encouraging tumor-suppressive capacity [18, 81–86]. In addition, it has been suggested that there might be an interaction between the immune system and chemotherapy leading to improved survival of CRC patients [55]. Therefore, an increased integration of immunotherapy into clinically approved concepts of standardized treatment of CRC can be expected in the near future [18].

Forkhead Box P3-Positive Regulatory T Cells ($FOXP3^+$Tregs)

The human immune system consists of an elegant balance between immune surveillance and immune tolerance of self-antigens. Recent evidence has revealed the existence of a unique $CD4^+$ T cell population, designated regulatory T cells (Tregs), as an important suppressor T cell population. Tregs play an important role in the prevention of autoimmune disorders by controlling the activity of autoreactive T cells. On the other hand, in the 1980s, several investigators already proposed an involvement of suppressor T cells in the immune tolerance against cancer. Tregs were originally identified as $CD4^+$ T cells that constitutively expressed the IL-2 receptor α-chain (CD25). Recent studies have shown that the transcription forkhead box P3 (FOXP3) is not only a key intracellular marker but also crucial for the development, maintenance and function of $CD4^+CD25^+$Tregs [61, 96]. Therefore, it is now generally considered that $CD25^+FOXP3^+CD4^+$ T cells are Tregs. Tregs are considered to act as players in immune tolerance against self-antigens, like most tumor-associated antigens (TAAs). This means that TAAs themselves may induce the increased number of Tregs seen in the peripheral blood or in the tumor tissue of varying types of tumor, including CRC, supporting a role for Tregs in cancer-induced immunosuppression [61, 96]. Tregs were associated with adverse outcomes in ovarian, breast, hepatocellular and gastric carcinomas. However, conflicting data exist in ovarian cancer and $FOXP3^+$TILs were not prognostic in renal cell carcinomas or in esophageal cancers. It is currently unknown as to whether Tregs can influence clinical outcomes in patients with CRC [96]. Overall, targeting Tregs may have an important impact on immunotherapeutic anti-cancer strategies and the clinical outcome of cancer patients [97].

$FOXP3^+$Tregs, Prognosis and MSI-Status

Several studies have been performed to gain an increased understanding of the role of Tregs in immune evasion and prognosis, whether or not according to MSI-status (Table 3). Loddenkemper et al. [97] reported that Treg density was lower in node-positive disease but was not associated with survival. In contrast, Ling et al. [98] found no significant difference in Treg density between advanced and early-stage disease, but did not evaluate the association with patient survival. Salama et al. [99] showed that a high density of $FOXP3^+$Tregs in CRC was associated with an improved survival and had a stronger prognostic significance than $CD8^+$ and $CD45RO^+$ memory T lymphocytes in CRC. In contrast, a high density of $FOXP3^+$Tregs in normal colon of CRC patients was associated with worse prognosis. The poorer outcome observed for these patients might be explained by the proposed role for these cells in suppressing antitumor immunity. However, the observation of better survival for patients with a high density of $FOXP3^+$Tregs in their tumor tissue is counter-intuitive and contrasts with what has been reported for other solid tumor types where Tregs are generally considered to be immunosuppressive. Suzuki et al. were not able to show a significant correlation between $FOXP3^+$Tregs and survival but they found evidence that a balance of intratumoral Tregs and $CD8^+$ T cells is a more sensitive predictor of recurrence and survival than intratumoral Tregs or $CD8^+$ T cells alone [61]. Similar results were also shown by others [96, 100]. Sinicrope et al. [96] also suggested that T cells may have distinct roles depending on their localization, which may contribute to the lack of prognostic significance of stromal $FOXP3^+$ or $CD3^+$ T cells in their study. Functional studies of $FOXP3^+$Tregs in cancer and normal tissue may shed more light on their role in the antitumor response and help to explain the observed associations with

Table 3 Role of FOXP3+ Treg cells in colorectal tumors. (CD: cluster of differentiation, CRC: colorectal cancer, DFS: disease free survival, ELISA: enzyme-linked immunosorbent assay, FACS: fluorescence-activated cell sorting, FOXP3: forkhead box P3, IHC: immunohistochemistry, IL-6: interleukin-6, IL-17: interleukin-17, it: intratumoral, MC: mast cells, MMR: mismatch repair, qRT-PCR: quantitative real time polymerase chain reaction, TAA: tumor associated antigen, TGF-β: transforming growth factor beta, TMA: tissue micro-array, Treg: T regulatory cell, VEGF: vascular endothelial growth factor)

Author	Investigated cell type	Methods	Conclusions
Loddenkemper et al., 2006 [97]	FOXP3 Treg	IHC	A direct link between Treg infiltration in the tumor and the development of a systematic T cell response in CRC cannot be proven. However, local Treg infiltration was significantly higher n limited disease, in which a systemic TAA-direct T cell response is less frequently observed.
Clarke et al., 2006 [33]	CD4CD25FOXP3 T cells	Flow cytometry	Tregs capable of inhibiting tumor associated antigen-specific immune responses are enriched in patients with CRC. These results support a rationale for manipulating Treg to enhance cancer immunotherapy.
Ling et al., 2007 [98]	Treg		The proportion of Treg was significantly higher in the peripheral blood of CRC patients. There were also more Treg in tumor infiltrating lymphocytes. Treg from CRC patients were FOXP3 positive and suppressed the proliferation of autologous $CD4^+$ cells.
Le Gouvello et al., 2008 [101]	CD3, IL17, FOXP3 Treg, cytotoxic markers and inflammatory cytokines	qRT-PCR and IHC	Immune expression profiling in CRC displayed different patterns according to MMR status. Higher FOXP3, IL6, TGF-beta and IL17 expression is a particular determinant in MMR-proficient CRC.
Yaqub et al., 2008 [34]			
Michel et al., 2008 [92]	Treg	IHC	The elevated number of CD8-positive lymphocytes found in MSI-H CRC is paralleled by an enhanced infiltration with CD8-negative FOXP3-postive cells suggesting that FOXP3-positive cells may play a role in the regulation of the immune response directed against MSI-H CRC at the primary tumor site.
Salama et al., 2009 [99]	FOXP3 Treg, CD8, CD45R0	TMA and IHC	Treg density in normal and tumor tissue had stronger prognostic significance than $CD8^+$ and $CD45R0^+$ lymphocytes. The finding of improved survival associated with high density of $FOXP3^+$ Tregs in CRC contrast with several other solid tumors.
Bonertz et al., 2009 [104]	Treg and T effector cells and broad panel of long synthetic peptides of defined tumor antigens	IFN-gamma ELISPOT assay, flow cytometry and Treg specificity assay	Treg exert T cell suppression in an antigen-selective manner. There were differences in the repertoires of antigens recognized by Tregs and effector/memory cells in the majority of CRC patients. The selection of antigens according to preexisting T cell responses may improve the efficacy of future immunotherapies for cancer and autoimmune disease.
Chaput et al., 2009 [105]	FOXP3 Tregs	Flow cytometry	The newly identified $CD8^+FOXP3^+$ T regulatory T cell population in CRC demonstrates strong immunosuppressive properties in vitro. These data suggest that these cells may contribute to tumoral immune escape and disease progression.
Sinicrope et al., 2009 [96]	CD3, FOXP3	IHC	A low intraepithelial $CD3^+/FOXP3^+$ cell ratio and reduced number of $CD3^+$ T cells were associated with shorter patient survival time, indicating the importance of an effector to Treg cell ratio in colon cancer prognosis.
Wada et al., 2009 [106]	Treg, VEGF	In vitro assay	VEGF contributes to the induction and maintenance of Tregs in patients with colon cancer. The percentage of Tregs in peripheral blood mononuclear cells can be reduced by administration of intravenous injection of bevacizumab.
Suzuki et al., 2010 [61]	CD8, FOXP3	IHC	$ItCD8^+T/itFOXP3^+$ cell ratio is a predictive marker for both disease free time and overall survival time in patients with

Table 3 (continued)

Author	Investigated cell type	Methods	Conclusions
			CRC. Tumor producing TGF-beta may contribute to the increased number of FOXP3$^+$ cells.
Blatner et al., 2010 [107]	Treg and MC	IHC, ELISA, FACS	MC induce Treg to switch function and escalate inflammation in CRC without losing T-cell-suppressive properties. IL6 and IL7 are not needed in this process.
Frey et al., 2010 [103]	FOXP3 T regs	TMA	High frequency of tumor-infiltrating FOXP3$^+$ T reg is associated with early T stage and independently predicts improved disease specific survival in MMR-proficient CRC patients.
Lee et al., 2010 [108]	CD3, CD45R0, FOXP3 and CD25	IHC	CD45R0$^+$ and FOXP3$^+$ TILs demonstrated independent prognostic significance for survival in the current investigation (stromal CD45R0 was significant prognostic factor for OS and DFS, while intraepithelial FOXP3 was a significant prognostic factor for DFS).
Correale et al., 2010 [109]	FOXP3 Treg	IHC	A higher FOXP3$^+$ T lymphocyte tumor infiltration score is a favorable prognostic factor in colon cancer patients undergoing chemo or chemoimmunotherapy.
Deng et al., 2010 [110]	FOXP3$^+$ Tregs, CD8$^+$ T cells	Flow cytometry	The frequency of FOXP3$^+$ Tregs in tumor draining lymph nodes may provide a valuable prognostic tool in the treatment of CRC.

prognosis. Although further studies are required before changes in clinical practice can be recommended, the results of Salama et al. suggest that the assessment of FOXP3$^+$Treg density in tumor and normal colorectal tissue in combination with vascular and perineural invasion could improve the prognostication of early stage CRC [99].

Few studies analyzed the immune gene expression profile of FOXP3$^+$Treg cells according to MMR status. Le Gouvelle et al. [101] observed that the MSI-H phenotype was associated with a higher expression level of the cytotoxic molecule perforin, whereas the MSS phenotype corresponded to higher levels of FOXP3 and several proinflammatory cytokines, especially IL-17. Furthermore an increase in the transforming growth factor β (TGF-β) was observed in MMR gene-proficient tumors. Taken together, the study by Le Gouvelle and co-authors provides indirect evidence for discriminatory differences in the host immune response in relation to the MSI phenotype in CRC [102]. In addition, Frey et al. demonstrated that a high frequency of tumor infiltrating FOXP3$^+$ Tregs is associated with early T stage and independently predicts improved disease specific survival in MMR-proficient CRC patients but not in MMR-deficient patients [103]. To clarify the significance of the reduced FOXP3 expression found in most MSI-H tumors, distinction between thymus-derived "natural" Treg cells with stable FOXP3 expression and induced T effector cells with transient FOXP3 expression will be required, as the latter may not be associated with the same suppressive capacity as "natural" Treg cells. The induction of transient FOXP3 expression in T effector cells might be augmented by high TGF-β levels present in the microenvironment. On the other hand, TGF-β together with IL-6 induces the generation of TH-17 cells, which are highly proinflammatory and have been reported to promote angiogenesis and tumor growth. In addition, another possible explanation could be that many of the TAAs in MSS CRCs represent self-antigens and may elicit a different Treg response from that of the "abnormal peptides" that are presumably generated by MSI cancer cells [102].

In contrast, Michel et al. [92] were the first to show that the density of FOXP3$^+$Treg cells infiltrating CRC were significantly higher in MSI-H tumors using immunohistochemistry, paralleling the enhanced number of CD8$^+$ cells in these tumors. In addition, Sinicrope et al. [96] showed that the intraepithelial to stromal ratios for FOXP3$^+$ and CD3$^+$ T cells were increased in MMR-deficient versus MMR-proficient colon cancers, yet the CD3$^+$/FOXP3$^+$ ratio were similar in both groups and the prognostic impact of this ratio was maintained in the full study cohort.

This discrepancy might be attributed to the different methodologies applied in these studies or the role of Treg cells may differ according to the localization of the infiltrating cells, the clinical stage or the genetic background of the tumors [103]. Further research is recommended to elucidate the role of FOXP3$^+$Treg and MMR status in CRC.

Conclusions

Although CRC has long been viewed as a poorly immunogenic tumor, the evidence reviewed here suggests that there is a significant host response to this disease and

that the presence of this response is associated with improved prognosis, suggesting that it may alter the natural history of the disease [13]. It has been accepted that MMR-deficient (versus MMR-proficient) CRCs show an enhanced immunogenicity with increased intraepithelial lymphocytes that may contribute to their favorable clinical outcome [96]. $CD8^+$ T cells are the main effectors of direct tumor cell lysis, but are nevertheless strongly dependent on an efficient interaction with highly specialized antigen presenting cells. The complex scenario of antitumor immune response is further influenced by the intratumoral presence of $CD45RO^+$ memory T cells, which appear to be critically involved in the control of tumor recurrence, and by the inhibitory and tumor-supporting function of regulatory T cells and possibly other cancer immune escape mechanisms [18]. In addition, it has been suggested that T cells may have distinct roles depending on their localization within the tumor (stromal versus intraepithelial). However, the published evidence is largely from retrospective analyses and requires testing in prospective clinical trials.

From a therapeutic point of view, this sophisticated interaction between activated immune cells and colorectal tumor cells provides a unique spectrum of potential targets for the development of innovative therapeutic strategies in the treatment of CRC. Immunological approaches to augment this host response are currently being tested in early-phase clinical trials and an increased integration of immunotherapeutic attempts into the clinically approved therapeutic concepts for standardized treatment of CRC can certainly be expected in the future.

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