

Colorectal polyposis and immune-based therapies

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The progression from precancerous (adenomatous) colon polyps to malignant colorectal cancer involves the complex actions of various cytokines on T cell proliferation, cell-cell adhesion, apoptosis and host immunity. A broad spectrum of new treatments, including innovative molecular therapies such as gene therapy and treatment with cytokines, is under experimental and preclinical investigation. Nonsteroidal anti-inflammatory drugs and selective cyclooxygenase-2 inhibitors have traditionally been used as inflammation-reducing agents in cases of colon adenoma. Currently, adjuvant immunotherapies such as recombinant gene therapy and antibody-cytokine fusion proteins are assuming a more significant role in the management of colorectal neoplasia. Furthermore, advances in antitumour necrosis factor antibodies for the treatment of ulcerative colitis and Crohn's disease may have potential as chemoprotective agents for the treatment of colon polyposis. The present review aims to discuss the immunological mechanisms underlying colon tumour progression and the molecular and immune-based therapies that are leading to new methods of prognosis and treatment.

Key Words: *Adenomatous polyps; Angiogenic; Antibody-cytokine fusion proteins; Apoptosis; Colorectal cancer; Cytokines; Integrin*

Adenomatous polyposis of the colon and colorectal cancer are diseases in which specific antigens or cytokine receptors are expressed on the cell surface of the tumour. Recently, there has been much investigation into the immunosuppressive and molecular-based events that allow colon adenomas to grow into metastatic lesions. Despite the actions of T helper cells TH1 and TH2 immune responses to foreign antigens, colorectal cancers are no less capable of invading and overwhelming host defences. There are several possible hypotheses explaining how tumour cells evade host immune responses, the most crucial of which involve cytokine receptors, death factors, antibody-targeted superantigens, tumour-infiltrating T cells and anticytokine antibodies. The role of cytokines in the immunological response is critical to an understanding of the malignant disease process and the mechanisms of action of antitumour therapies. The present review looks at the role of cytokines in host-tumour interactions and reviews the uses of cytokine treatments and immunotherapy in treating colorectal cancer.

La polypose colorectale et les immunothérapies

La progression de polypes coliques précancéreuses (adénomateuses) à un cancer colorectal malin met en jeu les actions complexes de diverses cytokines sur la prolifération des lymphocytes T, de l'adhésion entre cellules, de l'apoptose et de l'immunité de l'hôte. Un vaste spectre de nouveaux traitements, y compris des thérapies moléculaires novatrices comme la génothérapie et un traitement aux cytokines, fait l'objet de recherches expérimentales et précliniques. Les anti-inflammatoires non stéroïdiens et les inhibiteurs sélectifs de la cyclo-oxygénase 2 sont généralement utilisés pour réduire l'inflammation d'adénomes coliques. En ce moment, des immunothérapies adjuvantes telles que la génothérapie recombinante et les protéines de fusion anticorps-cytokines assument un rôle plus important dans la prise en charge de la néoplasie colorectale. De plus, les progrès réalisés à l'égard des anticorps du facteur de nécrose antitumorale pour le traitement de la colite ulcéreuse et de la maladie de Crohn pourraient avoir du potentiel comme agents chimio-protecteurs dans le traitement de la polypose colique. La présente analyse traite des mécanismes immunologiques sous-jacents à la progression de la tumeur colique ainsi que des thérapies moléculaires et des immunothérapies qui entraînent de nouvelles méthodes pronostiques et thérapeutiques.

Most colon cancers arise within pre-existing polyps or adenomas. Cytokines, angiogenic factors and matrix proteins are thought to be the driving force in the gradual transformation of a premalignant polyp to a cancerous lesion (1,2). Sporadic colorectal cancer develops from precancerous adenomatous polyps due to somatic gene mutations that can occur over a period of years or decades (3). In the presence of precancerous polyps, interstitial fibroblasts in the normal colon are transformed into myofibroblasts (4). Myofibroblasts are distinct cells that have the ability to secrete cytokines, chemokines, prostaglandins (PGs) and growth factors, and are considered critical in augmenting inflammation and neoplasia.

CYTOKINES AS IMMUNE MEDIATORS

In recent years, much information has come to light on the molecular and immunological basis of tumour-host interactions. The existence of an immune response to cancer, once considered implausible, is now widely supported by studies of

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immunological treatments in humans and transplantable murine cancer models in animals. Currently, antigens are being characterized that are involved in the immune recognition of human cancers, and antitumour T cells are being manipulated in patients to promote cancer regression. Experimental studies have shown that cell-mediated rather than humoral responses are responsible for the rejection of a transplanted tumour (5). The immune response is initiated when either cytotoxic T lymphocyte (CTL) CD8⁺ cells or CD4⁺ T helper cells recognize the antigen from a human cancer cell. These T lymphocytes bind to small molecules and specific peptides in the groove of the surface human leukocyte antigen and begin an immunological cascade designed to destroy the cancer (6). Previously, knowledge of human cancer antigens focused on the identification of antigens derived from human melanoma. However, the use of cDNA libraries to transfect fusion vectors into antigen-presenting cells has led to the identification of many new human tumour antigens that are recognized by T cells.

The acquired immune response to foreign antigens involves the production of effector cells through a process of activation, proliferation and differentiation. T cell activation requires signal transduction through several distinct cell-surface receptors. Most T helper lymphocytes require a co-stimulatory signal from the antigen-presenting cells to produce cytokines that can activate CTLs. Triggering the CD8⁺ activation pathway of TH1 helper cells produces a dramatic increase in proinflammatory cytokines, which then act on effector molecules to carry out antitumour activity. Two of the most crucial of these cytokines are interleukin (IL)-2 and IL-12, both of which function in the CD28-CD80 receptor-ligand system to promote antitumour cytotoxic responses (7,8). IL-12, a heterodimeric molecule composed of two bioactive chains, is secreted mostly by macrophages. B cells, monocytes, dendritic cells, Langerhans cells and keratinocytes may also secrete it. IL-12 plays an important role in developing the cytotoxicity of CTLs by upregulating the mRNA expression of the effector molecules granzyme (granzyme B) and perforin. IL-12 receptors are located on CD4⁺ and CD8⁺ T cells and, in addition to upregulating the activity of these cells, IL-12 induces the production of interferon-gamma (IFN- γ), a cytokine that functions with IL-12 to enhance T cell antitumour immunity. The role of IL-2 appears to act synergistically with IL-12 to upregulate natural killer (NK) cells and to substitute for CD8⁺ signalling during CTL development. IL-2 and IL-12 are able to enhance the ability of NK cells to lyse neuroblastoma and osteosarcoma cells *in vitro* and have been implicated in the regression of certain cancers (9,10).

In addition to IL-2, IL-12 and IFN- γ , much attention has been given recently to the antitumour immune effects of tumour necrosis factor-alpha (TNF- α) and the TNF-related apoptosis-inducing ligand (TRAIL). TNF- α is a well-known cytokine that has been implicated in a wide spectrum of diseases, including sepsis, diabetes, cancer, osteoporosis, rheumatoid arthritis, multiple sclerosis and inflammatory bowel disease (11). The cytokine, first described a century ago, is a homotrimer of 157 amino acid subunits, whose cDNA has been cloned and expressed to produce recombinant TNF- α . TRAIL, a more recently characterized member of the TNF superfamily, is a transmembrane protein that functions with the cytokine IFN- γ in antitumour immunosurveillance. Five receptors have been identified for TRAIL, though only two of

them are able to induce apoptotic signals. There are two TNF receptors, TNF-R1 and TNF-R2, with TNF-R1 triggering the majority of biological responses (12). The anticancer activity of TNF- α relies on a signal transduction pathway which involves the binding of TNF-R and TRAIL-R to their ligands, followed by the activation of several transcription factors including nuclear factor κ B and c-Jun, and the triggering of caspase-8 by fibroblast-associated (Fas) and Fas ligand (FasL) (13). Nuclear factor κ B promotes cell survival by blocking apoptosis and, in addition, can modulate the expression of several antiapoptotic molecules (14). TRAIL has exceptional antitumour potential due to its high cell-specificity in terms of induction of apoptosis and its ability to induce tumour regression without causing toxicity to other cells (15).

Whereas IL-2, IL-12, IFN- γ , TNF- α and TRAIL enhance the cytotoxic and apoptotic effects in response to malignancy, these actions may not be sufficient to eradicate invasive tumours. Many tumours survive and grow because normal cell types recognize and respond to them in preset patterns. Transforming growth factor-beta (TGF- β) belongs to a cytokine family of TGFs with profound effects on cell proliferation, differentiation, cellular adhesion, inflammation and host immunity (16). Increased levels of TGF- β have been observed in highly metastatic cells of murine carcinoma compared with less aggressive sub-lines, supporting the view that TGF- β is pro-oncogenic (17). TGF- β can act as a potent inhibitor of immune function and is thought to provide tumour cells with an escape from immune surveillance by allowing immunological tolerance of tumour antigens. The role of TGF- β in colon cancer may be related to mechanisms which control precursor TGF- β protein expression at the level of pretranslation of the TGF- β transcript or at the level of post-translation of the TGF- β protein. This process may be related to carcinogenesis and suggests that the suppression of the precursor TGF- β is an early event in the human colorectal adenoma-carcinoma sequence (18). Increased expression of TGF- β has been associated with malignant progression and conversion in colon, gastric, ovarian, and cervical cancers and in certain gliomas and melanomas, further supporting the pro-oncogenic role for TGF- β (19).

Paradoxically, TGF- β is a potent growth inhibitor in normal epithelial tissues, and is therefore considered highly protective in certain early stages of cancer. Several studies have implicated TGF- β in tumour-inhibitory processes such as genomic stability, induction of senescence and prevention of unsuitable angiogenesis. Intense signals of TGF- β 1 mRNA and the protein have been detected in human colorectal adenomas, supporting the view that colon cancer cells are able to escape from TGF- β -mediated growth inhibition by down-regulation of TGF- β receptors (20). Increases in TGF- β 1 stimulate the recruitment of tumour-infiltrating lymphocytes to the neoplastic site and cause a direct inhibitory effect of rat TNF- α and IL-10 on tumour proliferation *in vitro*. These seemingly contradictory findings on the role of TGF- β may be explained by a clear site dependency and biphasic mechanism of action. TGF- β levels are lowest in the rectum and highest in the ascending colon. Low levels of TGF- β are associated with the development of adenoma and support the higher epidemiologic incidence of colon neoplasia in the distal colon (21).

Cytokines that have been less well characterized in the context of a cell-mediated immune response to tumourigenicity are IL-4, IL-13, IL-1 and IL-8. IL-4 and IL-13 are structurally

similar molecules that are produced by TH2 cells in response to antigen receptor binding. They may be induced by mast cells or basophils and the cytokines have commonly been associated with allergy, the humoral response and autoimmunity. The main role of IL-4 is to regulate TH2 development, whereas IL-13 mainly regulates mucus secretion and inflammation of the bowel (22). IL-1 is known to regulate the proliferation and differentiation of normal and malignant immune cells and also to play a role in angiogenesis and cellular growth (23). IL-1 promotes the adhesion of cancer cells to the endothelial lining of blood vessels and induces the expression of matrix metalloproteinases in several cell types. IL-1 has been implicated in the progression of several malignancies, including cancer of the liver, lung and bone marrow (24). IL-8 is a cytokine with chemotactic properties that is able to induce respiratory burst, and promote angiogenic effects, degranulation, and enzyme release in neutrophils. Two receptors for IL-8 have been identified, both of which are members of the G-protein receptor family and are expressed in neutrophils, T cells, monocytes, macrophages, fibroblasts and melanoma cells (25). IL-13 and IL-4 convert IL-8 into monocyte chemotactic agonists by upregulating receptor expression. IL-8 and related chemokines are thought to aid in recruitment and positioning of mononuclear phagocytes in TH2-dominated responses (26).

There is some evidence that cytokines exert their immunological effects through the initiation of the cyclooxygenase (COX) pathway. The COX isoenzymes COX-1 and COX-2 are key metabolic enzymes that convert arachidonic acid to PGs. Metabolites of arachidonic acid are essential for numerous immunological and biological processes, including ovulation, inflammation, platelet aggregation and angiogenesis (27). COX-1 is constitutively expressed and synthesizes cytoprotective PGs in the gastrointestinal tract. COX-2 is the product of a gene that is expressed in response to growth factors, tumour promoters, or cytokines (28). The enzyme is inducible by the ras and src oncogenic cytokines, as well as by IL-1, TGF- β and TNF- α (29). COX-2 rapidly exerts proinflammatory effects in response to intracellular and external stimuli. Inflammatory stimuli and cytokines induce increased levels of COX-2, which in turn results in the increased production of PGs (PGE₂, PGF₂). PGE₂ increases the blood flow at the site of inflammation, resulting in capillary leakage and the initiation of a cell-mediated immune response. COX-2 has been shown to contribute to T cell development by positively affecting the CD4⁺/CD8⁻ population and by inducing CD4⁺ thymocyte development (30). Overexpression of COX-2 is associated with the stimulation of cellular division and angiogenesis as well as the suppression of apoptosis in cultured epithelial cells (31,32).

PATHOGENIC MECHANISMS

Currently, there has been much interest in cytokines as immunological mediators in colon adenoma and colorectal cancer. Many studies have associated inflammation and cancer of the colon with the immune-mediated actions of cytokines, and several studies have linked cytokine production and premalignant polyps. Cytokine involvement in colon cancer may be characterized by four types of biological activities: growth and proliferation of T cells, dysregulation of cell-cell adhesion signals, induction of apoptotic pathways and TGF- β -mediated growth control of neoplastic tissue (33).

Proliferation of T lymphocytes

Studies on the immune responses involved in colon cancer demonstrate that expression of cytokines secreted by TH1 cells can generate cell-mediated immunity against colorectal tumours. Colorectal cancer cells have the ability to downregulate T cell activity, with decreased T cell activation being found in the venous blood that drains from the tumour compared with arterial blood leading to the malignant lesion. IL-2-activated killer lymphocytes secrete inflammatory cytokines such as IFN- γ and TNF- α that can induce nitric oxide synthesis (34). Lymphocytes of patients with colorectal cancer are unable to develop into effective lymphokine-activated-killer cells or tumour-specific CTLs. IL-1 is known to regulate the proliferation and differentiation of immune cells in human colon cancer cell lines. IL-12 stimulates the proliferation of naïve T cells into TH1 cells and enhances the production of IFN- γ from activated T lymphocytes in vitro (35). IL-23, a novel cytokine that is structurally and functionally similar to IL-12, has been found to stimulate the proliferation of memory T cells and induce immune-dependent antitumour effects in murine colon carcinoma cell lines (36). In addition to the production of cytokines by TH1 cells, tumour cells themselves have been found to secrete immunosuppressive factors (ISFs) which inactivate tumour-infiltrating lymphocytes, leading to the suppression of immune defense. The role and scope of ISFs are not fully understood, though several ISFs have been identified at the molecular level as the cytokines TGF- β and C-reactive protein (37).

Dysregulation of cell-cell adhesion

There is growing evidence that the proinflammatory effects of some cytokines are attributable to their actions on cell-cell adhesion molecules. These adhesion molecules are associated with cytokine expression and may be involved in the migration of inflammatory cells toward the inflamed area. In particular, aberrances in the expression and function of integrins have been implicated in the tumorigenesis of colon cancer. Integrins are cell surface receptors which mediate the adhesion of cells to the extracellular matrix. In addition to their role as molecular 'glue', integrins allow nonmalignant cells to sense that they are attached to the extracellular matrix, thus providing a cell survival signal. This signal allows cells to proliferate in the presence of growth factors and in some instances prevents apoptosis (38). The ability to survive loss of cell-cell contacts is a marker of tumour progression and many adenomatous cultures survive only if cell-cell contacts are maintained. If cell-cell contacts are preserved, premalignant colonic epithelial cells are capable of survival in vitro (39).

Normal colonic epithelial cells are dependent on cell-cell adhesion and survival factors for the inhibition of apoptosis. In colorectal tumorigenesis, cells develop mechanisms to evade control signals and are able to survive despite the loss of cell-cell contacts. Overexpression of adhesion molecules and an abnormal enhancement of growth factors may be an indicator of colon tumorigenesis. In a recent study, chronic trinitrobenzene sulfonic acid-induced inflammation of the colon coincided with an increase in production of TNF- α , IL-1, IL-10 and IFN- γ , and expression of the adhesion molecules E-selectin, P-selectin, vascular cell adhesion molecule-1 (VCAM-1), intercellular cell adhesion molecules (ICAM-1, ICAM-2), focal adhesion kinase (FAK) and α 4 β 7 integrins (40). ICAM-2 is of particular interest as an antigen expressed in murine colon

cells which enhances leukotactic factor activity-1-independent adhesion of dendritic cells to the colon endothelium, thus increasing the blood supply for tumour angiogenesis (41). Similarly, FAK is involved in the promotion of cell survival under certain in vitro conditions and is observed to be over-expressed in invasive and metastatic colon cancer cell lines (42). The oncogenic effects of FAK are hypothesized to result from increased cell migration and increased cell survival under anchorage-independent conditions (43). In colon cancer cells, elevated levels of the cytokine Src cause the components of adherens junctions, including vinculin, to be redistributed to Src-induced integrin adhesion complexes, which blocks proper assembly of cell-cell contacts (44). Cytokines that have been found to inhibit cell-cell adhesion in colon tissue include the TH2 cytokines IL-4 and IL-13. Specifically, IL-4 and IL-13 are thought to inhibit cell-cell adhesion by downregulation of the adhesion molecule E-cadherin (45).

INDUCTION OF APOPTOTIC PATHWAYS

Apoptosis is an important regulatory mechanism by which unwanted cells are eliminated in tumorigenesis. Apoptosis is initiated by binding of death receptors such as Fas, TNF-R and TRAIL-R to their ligands. Binding of the receptors results in transmission of apoptotic signals and the activation of enzymatic caspase complexes (46). IFNs function in the apoptotic pathway by sensitizing cancer cells to apoptotic factors and inducing production of nitric oxide. The execution phase of apoptosis requires the participation of caspase proteases, which exert their downstream effects through a process of destruction of nuclear lamina, DNA fragmentation, membrane blebbing and cell shrinkage. Disassembly of the cell results in membrane-enclosed vesicles (apoptotic bodies), which are cleared by phagocytes without inciting an inflammatory response.

To clarify the role of apoptosis in the pathogenesis of colon cancer, apoptotic indices and expression of apoptosis-related antigens in colon tumour cells have been investigated. Fas, a member of the TNF/nerve growth factor receptor superfamily, mediates apoptosis in response to agonistic antibodies or FasL binding. Several studies report that FasL expression is significantly elevated in advanced colon and gastric carcinomas than in early adenomas, and that higher levels of FasL are correlated with increased apoptotic indices (47,48). The results indicate a strong role for Fas in the cancer-protective apoptotic activity of the colon.

TNF- α and TRAIL induce apoptosis in tumour cells of the colon but not in normal cells. Culture medium from macrophages of colon cancer patients was found to contain TRAIL at significantly higher levels than normal, indicating the significance of these cytokines in colon tumour cell apoptosis (49). A study by Dong et al (50) has provided further evidence that attenuation of apoptosis may facilitate tumour progression in colon cells. Dong et al examined the role of cytosolic phospholipase A2 (cPLA2) in TNF- α -induced apoptosis of cultured mouse colonocytes. The specific cPLA2 inhibitor, AACOCF-3 (arachidonoyl trifluoromethyl ketone), was able to protect colonocytes from TNF- α -induced apoptosis in vitro. The study supports the idea that down-regulation of cPLA2 may attenuate TNF- α mediated apoptosis during tumorigenesis and facilitate progression of colon carcinoma. Increased serum concentrations of soluble TNF-R1 have been found in noncachectic and cachectic patients with

advanced colorectal cancer (51). Postoperative plasma soluble TNF-R levels were also significantly elevated in postoperative analysis of patients who had undergone resection of colorectal carcinoma (52).

Tumour growth control and TGF- β

Recently, there has been much investigation into the effects of alterations in the TGF- β pathway in the pathogenesis of colorectal cancer. TGF- β inhibits growth and induces apoptosis of colon epithelial cells. The binding of TGF- β to its receptor induces phosphorylation of the Smad proteins Smad2 and Smad3, which then form heteromeric complexes with Smad4, travel to the nucleus, and activate gene transcription. *Smad4* is a tumour-suppressor gene that controls cell growth. *Smad4* activity is required for TGF- β signaling, and *Smad4* mutations present in some cancers have been considered sufficient to inactivate TGF- β signalling (53). One study examined the coding regions of DNA from 100 patients with colon cancer and reported that abnormalities in the TGF- β type II receptor and Smad4 played an important role in inhibiting TGF- β signalling in colorectal carcinogenesis (54). Enhanced Smad4 protein expression has been closely linked to overexpression of TGF- β II receptors in hepatocellular carcinoma and a loss of Smad4 has been associated with carcinoma in a number of other organs, including the pancreas and colon (55).

LIPOPOLYSACCHARIDES AND INFLAMMATION OF THE COLON

The role of lipopolysaccharide (LPS) in contributing to inflammation of the colon is currently uncharacterized. Both normal colon epithelium and colon carcinoma cells in situ are continuously exposed to LPS. In a study by Strassman et al (56), the involvement of IL-6 was examined in a LPS challenge. The administration of LPS to mice induced a transient weight loss, hypoglycemia and an increase in fibrinogen. Administration of the IL-6 antibody resulted in a significant improvement of LPS-induced hypoglycemia and weight loss as well as a significant decrease of plasma fibrinogen. Pretreatment with TNF antibody was able to completely inhibit elevation of triglycerides and modestly improve LPS-induced weight loss. These results suggest that IL-6 and TNF- α , two cytokines implicated in colon diseases, are also involved in reversing metabolic changes associated with LPS-induced inflammatory conditions of the intestine. Few reports have addressed possible direct effects of LPS in the promotion of adenomatous polyps to colon carcinoma. Kojima et al (57) found evidence that LPS directly stimulated growth of the human colon carcinoma cell line through an increase in the production of PG. LPS, a bacterial endotoxin in the gut, may stimulate DNA synthesis in certain colon carcinoma cells. Further investigation of the pathways mediating LPS-induced stimulation of colon carcinoma cells may provide insights into the mechanism by which in vivo adenomas are transformed to cancer.

The effect of diet on colon cancer is thought to be mediated through insulin-like growth factor (IGF), a molecule produced and secreted by the liver. Epidemiological studies have shown that IGF-I is positively associated with the risk of colorectal cancer, and experimental studies have shown that IGF-I has mitogenic and antiapoptotic actions on colorectal cancer cells (58,59). IGFs I and II and their principle receptor, IGF-I receptor (IGF-IR), are frequently expressed in human colon cancers

and play a role in preventing apoptosis, enhancing cell proliferation and inducing expression of vascular endothelial growth factor (VEGF) (60). Colorectal epithelia display IGF-1 receptors *in vitro*, and when bound to IGF, the receptor-ligand complex inhibits apoptosis and allows progression through the cell cycle (61). Clinical conditions associated with high levels of insulin and IGF-1 (such as diabetes and hypertriglyceridemia) are related to an increased risk of colon cancer and colonic neoplasia (61). IGF-1 is known to regulate the mitogenic properties of insulin in normal and neoplastic colon cells (62,63). The association between IGF and cytokines is in the early stage of investigation, but at least one study has shown that IGF-1 is capable of producing total resistance of colonic tumour cells to TNF- α and IFN- γ -mediated apoptosis (64).

EMERGING THERAPIES

Nonsteroidal anti-inflammatory drugs and COX

The use of immunotherapy to treat colon polyps has been extensively studied by many investigators and has yielded some promising results. The correlation between neoplastic colon tumours and increased levels of PGs led to the investigation of the use of nonsteroidal anti-inflammatory drugs (NSAIDs) as potential therapeutic agents. Currently, there is a large body of epidemiological and clinical evidence demonstrating the effectiveness of NSAID therapy in the prevention and treatment of polyposis and colorectal cancer. Experimentally, it has been shown that the effectiveness of NSAIDs in treating adenomatous polyps and colon cancer results from the inhibition of COX-2 (65). COX-2 is overexpressed in 50% of benign polyps and 80% to 85% of adenocarcinomas. NSAIDs are thought to affect the COX system primarily by restoring apoptosis and reducing tumour growth and angiogenesis (66).

The expression of COX-2 is strongly implicated in polyp formation in adenomatous polyposis coli (APC)-mutated mice models. Treatment of APC mice with sulindac inhibited tumour formation, decreased COX-2 and PGE₂ expression in the small bowel, and restored apoptosis to baseline levels (67). In cell populations of precancerous familial adenomatous polyposis (FAP) colonic mucosa, expression of COX-2 among FAP colonic mononuclear and lateral crypt epithelial cells was increased four-fold compared with controls (68). In a mouse model for human FAP, deletion of the gene encoding a cell-surface receptor of PGE₂ resulted in a decrease in the number and size of intestinal polyps in human adenomatous polyposis (Apc) formation mice (69). Overexpression of human PGE synthase, an enzyme which converts PG to PGE₂, is thought to contribute to increased amounts of PGE₂ in colorectal adenomas and cancer. The experimental evidence suggests that PGE₂ plays a major role in mediating COX-2 upregulation in intestinal polyps, and that the effects of PGE₂ may be blocked by various anti-inflammatory agents. Treatment with the specific COX-2 inhibitor rofecoxib results in a dose-dependent reduction in the number and size of intestinal and colonic polyps in the APC-infected mouse (70). Polyps from either rofecoxib- or sulindac-treated mice show lower rates of DNA replication, express fewer proangiogenic vascular endothelial-derived growth factors and more membrane-bound beta-catenin. The inhibition of polyposis in the mouse model suggests that the specific COX-2 inhibitor rofecoxib (Vioxx, Merck Frosst Canada Inc, Canada) has similar therapeutic potential as sulindac in preventing human intestinal and colon cancer.

In support of the experimental evidence, clinical studies report that chronic ingestion of nonsteroidal anti-inflammatory medication is able to diminish or, in some cases, reverse the development of polyps in the colon. The use of sulindac in FAP patients has shown a statistically significant decrease in the number and size of polyps (71). Numerous retrospective and prospective studies involving the use of acetylsalicylic acid (ASA) in humans have shown a reduced risk of developing colon cancer in individuals who took these drugs (72-74). Sandler et al (75) have demonstrated a protective effect of ASA in a population of patients who had previous colorectal cancer and were at risk for developing sporadic polyps. The introduction of the selective COX-2 inhibitors such as celecoxib and rofecoxib are the most recent drugs being investigated as chemopreventive agents in colorectal polyposis because they are associated with reduced gastrointestinal side effects of NSAIDs (76,77). Although selective COX-2 inhibitors represent an improvement over nonselective NSAIDs, they do not completely eliminate gastrointestinal toxicity or the renal side effects associated with use of conventional NSAIDs (78). COX-2 selectivity has also raised concerns regarding the risk of cardiovascular complications and prothrombotic events, though there is no conclusive evidence that COX-2 inhibitors lead to vascular ischemia (79).

Clinical studies of ASA use have been useful in establishing the chemoprotective effects of downstream cytokines in colorectal cancer patients. In one study, the effect of ASA on the production of cytokines TNF- α and IFN- γ was studied in six healthy volunteers (80). Four days after cessation of a three-day regimen of 650 mg of ASA, there was a 70% increase in whole blood IFN- γ production ($P < 0.05$). In addition, there was a four-fold increase in the production of TNF- α ($P < 0.03$). Short term ASA treatment is thought to induce an increase in the production of these cytokines, probably through inhibition of PGs. The data reveal a novel pathway through which ASA may modify apoptotic effects in the development of colon tumours. In a similar investigation, treatment with 81 mg of ASA per day for three months altered the expression of TGF- α in normal-appearing rectal mucosa from individuals with a history of adenomatous polyps (81). The observation that ASA reduced rectal mucosal PGE₂ formation and TGF- α expression in patients with a history of adenomatous polyps suggests a possible future role for TGF- α in treatment of colon adenoma.

Anti-TNF therapy and integrin antagonists

Several novel immunosuppressive therapies involving anti-TNF biologics and integrin blockers have shown promising results for the treatment of polypoid dysplastic lesions associated with Crohn's disease (CD) and ulcerative colitis (UC) (82). Because CD and UC have similar morphological and pathological features to colon adenoma, it can be expected that these therapies will be additionally effective in treating sporadic or neoplastic polyps. CD is associated with a TH1 immune response in the layers of the gut whereas mucosal inflammation in UC is hypothesized to occur through a TH2 type response (83). In chronic intestinal lesions in CD, TH1 cytokines such as IFN- γ , TNF and IL-12 are predominant (84). The presence of neoplastic polyps in patients with UC and CD is associated with an increased risk of invasive colon carcinoma (85). In clinical trials with CD patients in particular, anti-TNF antibodies and 4-integrin antagonists are beginning to target

specific pathogenic pathways, leading to prolonged remission of the polyp-initiated inflammatory process (86).

The standard of anti-TNF therapy is infliximab (Remicade, Schering Canada Inc, Canada), a chimeric monoclonal antibody which is approved by the US Food and Drug Administration (FDA) for the treatment of CD. Infliximab has been shown to bind to soluble and membrane-bound TNF, thereby neutralizing TNF- α and restoring the immunological imbalance of the inflamed gut mucosa. Several clinical trials have shown the value of infliximab for the induction of remission of refractory and fistulizing CD (87,88). Clinical studies with infliximab suggest that treatment results in a rapid and thorough inhibition of clinical signs and symptoms (89,90). Infliximab is thought to be effective in approximately two-thirds of patients with UC or CD-associated colitis (91). The dramatic clinical effect of infliximab probably results from the fact that after binding of membrane TNF, the drug induces cytolysis of the inflammatory cells by antibody-dependent cytotoxicity (84). This may lead to clonal depletion of T cells expressing TNF. Moreover infliximab promotes apoptosis of activated T cells by increasing the ratio of Bax to Bcl-2 (92). Treatment with infliximab is associated with many alterations in immune and inflammatory pathways, which are attributed to TNF blockade. The expression of adhesion molecules such as E-selectin, ICAM-1 and VCAM-1 appear to be down-regulated following administration of infliximab and tissue levels of inflammatory cytokines such as IFN- γ , IL-1 and IL-6 are reduced (93). Besides its known TNF- α neutralizing property, infliximab downregulates INF- γ production in colonic T cell cultures (94).

In addition to the "biologic" anti-TNF drug infliximab, progress has been made in CD with the recombinant humanized antibody natalizumab (Antegren, Elan, USA), which is an integrin antagonist that targets the alpha-4 (α 4) integrin molecule (95,96). Treatment with the selective adhesion-molecule inhibitor increased the rates of clinical remission and response, improved the quality of life and C-reactive protein levels, and exhibited efficacy in a Phase II, randomised, controlled trial of patients with CD (97,98). Natalizumab has not been shown to be effective in the treatment or remission of UC, though a single 3 mg/kg infusion of natalizumab was well-tolerated by UC patients (99,100). A large multicentre placebo-controlled study in 240 patients showed significant efficacy of natalizumab in the treatment of chronic active CD (101). The mechanism of action of natalizumab involves the blockade of the migration adhesion molecules to the inflamed gut (102). Lymphocyte infiltration into the intestinal tract in CD is mediated by the interaction between α 4 integrin, expressed on lymphocytes, and its ligand mucosal vascular addressin cell-adhesion molecule-1. Natalizumab blocks the migration of integrins to the site of mucosal inflammation, but it may also prevent the interaction between alpha-4 (α 4) integrin molecule and VCAM-1 (103).

Antiangiogenic therapy

Research into the role of endogenous angiogenic factors in cancer metastasis has led to the development of promising new antivascular therapies. VEGF is currently the best-characterized regulator of tumour angiogenesis and a main target of anti-tumour therapeutic approaches in breast, lung, renal and colon carcinomas (104). VEGF plays an important role in the maintenance of existing tumour vessels and is expressed in a range

of cells in response to cytokines, growth factors and cell-bound stimuli (105). The recently FDA-approved anti-VEGF monoclonal antibody bevacizumab (Avastin, Genentech Inc, United States) has been designed to block the action of VEGF, thereby decreasing tumour perfusion, microvascular density, and circulating endothelial and progenitor cells in cancer metastasis (106,107). The antibody is currently completing phase III trials to establish the efficacy of a 5 mg/kg dose of bevacizumab plus chemotherapy as first-line therapy for metastatic colorectal cancer (108,109). A review of 900 patients receiving Avastin plus 5-fluorouracil/leucovorin (5-FU/LV) showed that the combined therapy improved median survival by approximately five months, compared with patients treated with chemotherapy alone (Genentech Inc, December 2003). Avastin was well tolerated, though grade 3 hypertension and gastrointestinal perforations were reported as side effects in some patients. Targeting tumour vasculature is emerging as an important approach to managing colorectal cancer, because it increases the effectiveness of conventional chemotherapy, while offering physical accessibility and genetic stability of target cells.

Recombinant gene therapies

Gene therapy as an adjuvant cancer therapy is well established, with a number of trials ongoing and a vast range of other approaches being assessed in animal and cell culture experiments. Replication-deficient recombinant adenoviral vectors are predominantly used for colon cancer gene therapy, because they can be produced at high titer and they readily infect a number of different cell types (110). Gene therapy studies in the United States are currently passing into clinical trials. Phase I trials have demonstrated the safety of various delivery systems with respect to toxicity of adenovirus and side effects of transgenes, but have not succeeded in establishing significant therapeutic benefit (111). The insertion into solid tumours of genes encoding cytokines, which recruit inflammatory or immune cells to the sight of malignancy, is a potent strategy for enhancing the immunogenicity of tumours (112,113). Preclinical models have tested a range of cytokines including IL-2, IL-18, IL-12, IL-23, granulocyte macrophage-colony stimulating factor (GM-CSF) and IFN- γ (114,115). While *in vivo* models confirm that gene therapy is able to generate tumour-specific immunity following challenge with new, untransduced tumour cells, the treatment is less efficient in eradicating established tumours.

Gene therapy with IL-2 has shown antitumour efficacy in several murine models of colorectal cancer, and is being investigated in clinical trials that employ various reagents and delivery systems. In a phase I clinical trial, 10 patients with colorectal cancer were treated with IL-2 gene therapy (116). Autologous fibroblasts were transduced with a retrovirus carrying the IL-2 gene and mixed with autologous irradiated tumour cells before subcutaneous re-injection. In two of six evaluable patients, there was successful induction of tumour-specific CTL precursors and in five of 10 patients there was evidence of immunological memory by delayed-type hypersensitivity skin reaction to subsequent injection. In a similar experiment, autologous immune effector cells were transfected with the IL-2 gene. Ten patients (predominantly with colorectal cancer) were treated with autologous cancer cells transfected with the human IL-2 killer gene derived from peripheral blood mononuclear cells that had been transfected with an

IL-2 plasmid (117). Evidence of biological activity was indicated by an increase in serum IFN, GM-CSF and TGF- β during treatment and also an increase in the cytotoxic activity of circulating lymphocytes compared with human leukocyte antigen matched carcinoma cell lines.

IL-12 has been used to induce antitumour immunity in several experimental models of colon carcinoma and is currently being considered for application in humans. Mazzolini et al (118) have demonstrated the therapeutic usefulness of adenovirus-mediated transfer of the IL-12 gene in a murine model of colon cancer. Intratumoural injection of the IL-12 gene induced a local increase in IL-12 and IFN- γ levels and a complete regression of the tumour in 76% of mice. The antitumoural effect was mediated by CD8+ T cells and was associated with the production of CTLs directed against malignant cells. Animals that eliminated the tumour were protected against a second administration of neoplastic cells (118). In another experiment, the adenovirus-IL-2-induced immune response was shown to involve the infiltration of NK cells and mediation of tumour cell killing as early as 48 h after treatment (119). The antioncogenic properties of IL-12 may be enhanced by coadministration of a plasmid containing IFN- α . Experimentally, combination gene therapy of IL-12 with IFN- α synergistically increased the anti-tumour response in mice against the colon cancer cell line CT26. Upregulation of CD40 molecules on antigen-presenting cells was evident, though in vivo depletion of leukocytes indicated that CD8+ T and NK cells were the primary effectors of the antitumour response. Mice that rejected the primary tumours after combined treatment with IL-12 and IFN- α plasmid generated protective immunity against a subsequent tumour challenge, indicating a synergistic effect of the combination of cytokines on the regression of established tumours (120). In a study using the poorly immunogenic murine colorectal cancer cell line, MC38, injecting a combination of IFN- α /IL-12-transfected cells caused an additive antitumour effect therapy model (121). Using the same cancer cell line, Eguchi et al (121) and Kobayashi et al (122) confirmed the enhancement of MC38-specific cytotoxicity and a reduction in DNA fragmentation in CTLs when murine cancer cells were transduced with IFN- α . The prevention of apoptotic cell death in tumour-specific CTLs and the recruitment of CTLs appears to elicit long-lasting immunity that augments the nonspecific killing of IL-12.

Two other IFNs that have been studied for use in treating colon cancer are IFN- γ and IFN- β . Cytokines belonging to the IFN family possess pleiotropic biological activities that play important roles in tumour suppression and rejection and hence are especially promising as in vivo cytokine gene therapy targeted against metastatic colon cancer (122). Niv et al (123) investigated the effect of recombinant human IFN- γ on tumour-associated mucin antigens in HT-29 and LIM-6 colon cancer cell lines in vitro. Secretion and synthesis of mucins by malignant cells were induced by IFN- γ in a dose-dependent manner, confirming that recombinant human IFN- γ enhances the expression of histocompatibility antigens and certain tumour-associated antigens in several colon carcinoma cell lines (123). Clinically, Schwartzberg et al (124) designed a phase I trial to enhance the selective action of 5-FU/LV by IFN- γ in the treatment of colorectal carcinoma. Clinically relevant IFN- γ concentrations sensitized HT29 colon carcinoma cells in vitro to 5-FU/LV cytotoxicity, presumably by up-regulating Fas death receptor expression and

sensitizing human colon carcinoma cells to TRAIL-mediated apoptosis (124). In an experiment using the IFN- β variant, recombinant adenovirus containing the human IFN- β cDNA was delivered systemically in nude mouse xenograft models of human colorectal cancer liver metastases. The vector targeted hepatocytes that produced high levels of IFN- β in the liver, resulting in a significant apoptotic response followed by tumour regression. A similar recombinant adenovirus containing the murine IFN- β cDNA also resulted in a therapeutic response and improved survival in syngeneic mouse models of colorectal cancer liver metastases, implying that IFN- β gene therapy has potential for clinical evaluation as a long term cure for colorectal carcinoma (125). Lastly, human IFN- β was investigated in combination with 5-FU in vitro using the human colon cancer cell line C-1 and in vivo with the murine colon cancer cell line Co-4. When IFN- β was added to the tumour cells with 5-FU, the antitumour activity of IFN- β alone increased in a dose-dependent manner against Co-4 in nude mice, whereas its antitumour activity in vitro against C-1 was limited (126). The synergistic effect of 5-FU and IFN- β was observed to some degree both in vitro and in vivo and the clinical usefulness of this combination therapy for the treatment of advanced colorectal carcinoma is expected to pass to clinical trials in the near future. The antioncogenic effect of IFN- β appears to involve influx of activated NK cells, polymorphonuclear cells, and minimally-infiltrating macrophages. Antitumour effects of IFN- β may be different in different tumour types or in different anatomic locations, but it is established that tumour-specific CD4+ and CD8+ T cells are the key effector cells in tumour eradication using IFN- β gene therapy (127).

ANTIBODY-CYTOKINE FUSION PROTEINS

Advances in genetic engineering and expression systems have led to rapid progress in the development of antibodies fused to other proteins. Targeting of cytokines into the tumour microenvironment using antibody-cytokine fusion proteins, called immunocytokines, represents a novel approach in cancer immunotherapy. These 'antibody fusion proteins' include antibodies with specificity for tumour-associated antigens fused to cytokines such as IL-2, GM-CSF and IL-12 (128). Antibody-based therapies for cancer rely on the expression of defined antigens on neoplastic cells (129). The goal of this approach to cancer therapy is to concentrate the cytokine in the tumour microenvironment and thereby stimulate cellular T cell, B-cell, or NK immune responses against malignancies (130). In the past decade, multiple antibody-cytokine fusion proteins have been developed with different specificities targeting a broad variety of tumours. These novel molecules retain both antibody and cytokine associated functions and have been effective in eradicating established metastases in neuroblastomas, melanomas, and hepatic and pulmonary carcinomas in mouse models. Efficacy appears to be greatest when the mAb can recruit the effector cells of the host's immune system into helping in the mediation of the antitumour effect (131). In animals bearing malignancies, antibody-cytokine fusion proteins are able to elicit a significant antitumour response that in some cases results in a complete elimination of the tumour.

Current research in immunocytokines suggests that antibody-cytokine fusion proteins using IL-2 may have potential for use in the treatment of human colon cancer. Fusion protein-directed IL-2 therapy induces a T cell-dependent host immune

response capable of eradicating established metastatic lesions of the colon in animal tumour models. When IL-2 fusion proteins constructed of a tumour-specific antibody joined to IL-2 were used to deliver cytokine directly to the site of tumour cells in vivo, the protein was found to augment lysis of tumour cells. Analysis of the mechanism of cytotoxicity revealed that the fusion protein mediates the formation of stable conjugates between T cells expressing IL-2R and tumour cells expressing the specific antigen, resulting in lysis through the Fas-Fas ligand pathway (132). In one study, Xiang et al (133) used a recombinant humanized antibody-IL-2 fusion protein to direct IL-2 to the tumour microenvironment and elicit a T cell-mediated eradication of established pulmonary and hepatic metastatic lesions of the colon in syngeneic mice (133,134). The T cells that were isolated from tumour-bearing mice following fusion protein therapy elicited major histocompatibility complex class I-restricted cytotoxicity in vitro against colon carcinoma target cells. An increase in frequency of CTL precursors, induction of genes encoding TH1 cytokines, and the generation of primed tumour-specific CD8+ T cells culminated

in the complete rejection of the tumour cell challenge and the prevention of pulmonary metastasis.

CONCLUSION

Colorectal cancer is a significant contributor to morbidity and mortality in North America. Adenomatous polyps and early cancerous lesions can occur for some time before cancer is detected clinically. The large number of new cases of colorectal cancer that are diagnosed each year and the lack of adequate therapies underscore the need for novel immunotherapeutic approaches. Current research suggests that colorectal tumour establishment and progression involve a malfunction of the immune system. Elucidation of the role of cytokines and immune mediators in the progression of colon adenomas is leading to the development of anti-TNF antibodies, anti-integrins, antivasular treatments and recombinant gene therapies, all of which specifically target cytokine-mediated carcinogenic pathways. Knowledge of the mechanisms underlying inflammation of the colon in patients with adenomas will contribute to a greater understanding of colon cancer and improved methods of prognosis and treatment.

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