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Review

The relationship between angiogenesis and the immune response in carcinogenesis and the progression of malignant disease

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Abstract

Recent studies have demonstrated that angiogenesis and suppressed cell-mediated immunity (CMI) play a central role in the pathogenesis of malignant disease facilitating tumour growth, invasion and metastasis. In the majority of tumours, the malignant process is preceded by a pathological condition or exposure to an irritant which itself is associated with the induction of angiogenesis and/or suppressed CMI. These include: cigarette smoking, chronic bronchitis and lung cancer; chronic oesophagitis and oesophageal cancer; chronic viral infections such as human papilloma virus and ano-genital cancers, chronic hepatitis B and C and hepatocellular carcinoma, and Epstein–Barr virus (EBV) and lymphomas; chronic inflammatory conditions such as Crohn's disease and ulcerative colitis and colorectal cancer; asbestos exposure and mesothelioma and excessive sunlight exposure/sunburn and malignant melanoma. Chronic exposure to growth factors (insulin-like growth factor-I in acromegaly), mutations in tumour suppressor genes (*TP53* in Li Fraumeni syndrome) and long-term exposure to immunosuppressive agents (cyclosporin A) may also give rise to similar environments and are associated with the development of a range of solid tumours. The increased blood supply would facilitate the development and proliferation of an abnormal clone or clones of cells arising as the result of: (a) an inherited genetic abnormality; and/or (b) acquired somatic mutations, the latter due to local production and/or enhanced delivery of carcinogens and mutagenic growth factors. With progressive detrimental mutations and growth-induced tumour hypoxia, the transformed cell, to a lesser or greater extent, may amplify the angiogenic process and CMI suppression, thereby facilitating further tumour growth and metastasis. There is accumulating evidence that long-term treatment with cyclo-oxygenase inhibitors (aspirin and indomethacin), cytokines such as interferon-α, anti-oestrogens (tamoxifen and raloxifene) and captopril significantly reduces the incidence of solid tumours such as breast and colorectal cancer. These agents are anti-angiogenic and, in the case of aspirin, indomethacin and interferon-α have proven immunomodulatory effects. Collectively these observations indicate that angiogenesis and suppressed CMI play a central role in the development and progression of malignant disease.

Keywords: Angiogenesis; Immune response; Carcinogenesis; Malignant disease

1. Introduction

Angiogenesis plays a central role in ovulation, implantation of the fertilised ovum, fetal growth and gestation, and wound healing and repair following surgery and trauma [1]. These situations are paralleled by reduced cell-mediated immune responses (CMI) and upregulation of the humoral immune response (HI) [2–4] suggesting a close inter-relationship between the immune system and the angiogenic process in normal physiological processes.

The discovery of immune cytokines such as the interleukins (IL) and interferons (IFN) led to investigations of the types of cytokine produced by different immune cells in response to different antigenic stimuli. The results were complex and confusing until two main patterns of cytokine response in T-helper (CD4+) lymphocytes (Th cells) were identified [5]. Th1 lymphocytes synthesise IL-2, IFN-γ and tumour necrosis factor

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(TNF) in response to antigenic stimuli and are associated with CMI. These cytokines are part of the pro-inflammatory cytokine group which also includes IL-1α and IL-1β. IL-12, a more recently identified cytokine produced by macrophages and dendritic cells, plays a central role in CMI, inducing the conversion of Th0 cells to a Th1 phenotype. Th2 lymphocytes synthesise IL-4, IL-5, IL-6, IL-10 and IL-13. These are associated with the development of HI and, in general, are classified as anti-inflammatory cytokines [6,7]. The balance between CMI and HI is relevant to a range of human diseases. In particular, chronic infectious diseases such as tuberculosis [8], leprosy [9], leishmaniasis [10], hepatitis B and C [11–14] and HIV/AIDS [15,16] are characterised by loss of the CMI response associated with an overcompensated HI response. The impact of this polarisation of cytokine response in chronic disease, including malignancy, has led to new approaches in treatment such as boosting CMI responses with agents including IL-2, IFN-α and thalidomide [17–19].

2. Angiogenesis and malignant disease

Many malignant diseases, including those of the breast, lung, colon, cervix, bladder and skin, begin as *in situ* carcinomas. These tumours usually measure less than 1–2 mm in diameter and may be present for years until malignancy develops. Current opinion suggests that with time a subgroup of cells within the tumour takes on an angiogenic phenotype with the capacity to induce new blood vessel formation. As a result the disease develops the capacity to grow and metastasise. This is known as the 'angiogenic switch' theory. The cells in 'prevascular phase' neoplastic disease and in non-vascularised 'dormant' micrometastases from metastatic tumours may proliferate at the same rate as, or indeed more rapidly than, cells in well vascularised, enlarging, invasive primary and secondary tumours. However, in the absence of new vessel formation, the rate of proliferation of the tumour cells reaches equilibrium with the rate of apoptosis until such time as an angiogenic environment develops [1,20].

The presence and intensity of angiogenesis, as assessed indirectly by blood vessel counts, play an important role in the pathogenesis of malignant disease. High microvessel counts are an independent adverse prognostic factor in many solid tumours including breast [21], lung [22], prostate [23] and ovarian cancer [24] irrespective of the counting method employed, be it microvessel density, Chalkley count or computer image analysis [21].

However, a number of recent observations suggest that the presence of an angiogenic environment may precede, rather than precipitate or indicate the onset of, the development of frankly malignant disease. Increased angiogenesis compared with normal tissues is seen in a range of premalignant neoplastic conditions including metaplasia, dysplasia and carcinoma *in situ* of the lung [25,26] breast ductal carcinoma *in situ* (DCIS) [27] and cervical intraepithelial neoplasia (CIN) [28]. In the cervix, significant increases are seen in microvessel density from normal cervix through CIN I, II and III to invasive squamous cell carcinoma [28].

There is accumulating evidence that the angiogenic process may precede and participate in the process of carcinogenesis itself. Angiogenesis plays a significant role in the evolution of diseases associated with the subsequent development of malignant disease. These include chronic hepatitis and cirrhosis of the liver induced by hepatitis B and C [29,30] and pleural plaque formation following exposure of mesothelium to asbestos [31]. In these situations, the angiogenic process has been postulated to play a role in tumorigenesis leading, in susceptible individuals, to the development of hepatocellular carcinoma [14,29,32] and mesothelioma [31]. Furthermore, HIV and Kaposi's sarcoma-associated herpes virus (KSHV/HHV8) encode factors that promote angiogenesis and have been implicated in the development of Kaposi's sarcoma [33,34]. Angiogenesis is also increased in inflammation of the tunica mucosa in the lung [25], a process which may be increased by cigarette smoking [35].

A clear correlation has been established between the extent and intensity of expression of angiogenic growth factors and angiogenesis and prognosis in solid tumours [36,37]. Elevated plasma/serum levels of angiogenic growth factors may also correlate with microvessel counts within tumours as has been shown for transforming growth factor-beta 1 (TGF-β1) and hepatocellular carcinoma [38]. Induction of the expression of angiogenic growth factors in malignant cells is associated with an increased capacity of these cells to induce angiogenesis *in vitro* and *in vivo* [39]. Overexpression of angiogenic growth factors such as vascular endothelial growth factor (VEGF) and platelet-derived endothelial cell growth factor (PD-ECGF) is seen in premalignant neoplastic lesions such as CIN and DCIS [28,40,41]. PD-ECGF has been implicated in the process of remodelling of the pre-existing vascular network and in generating a dense rim of microvessels around breast DCIS [27].

However, the expression of angiogenic growth factors by epithelial tissues is not necessarily associated with either angiogenesis or neoplastic disease. Intense angiogenic growth factor expression, suggestive of deregulated synthesis, may be seen in dysplastic and metaplastic tissues which, in the majority of cases, regress spontaneously [41,42]. Serum levels of basic fibroblast growth factor (bFGF), a surrogate marker of angiogenesis, are similar in patients with cirrhosis of the liver and hepatocellular carcinoma [43]. Likewise serum

VEGF levels are similar in patients with benign and malignant lung disease [44]. Plasma VEGF levels are elevated in chronic hepatitis and cirrhotic liver disease [45] whilst serum VEGF levels are elevated in patients with acute hepatitis compared with healthy individuals [46]. Finally, in inflammatory bowel disease, inflamed mucosa of patients with active disease shows a significantly higher spontaneous production of VEGF by colonic mucosa than the normal mucosa of controls [47]. In keeping with this, serum VEGF levels are significantly elevated in patients with inflammatory bowel disease and reflect disease activity [48,49]. Furthermore, angiogenic growth factors such as VEGF and PD-ECGF are constitutively expressed by epithelial cells of the upper aero-digestive tract, including bronchial and bronchiolar epithelium, tongue and oesophagus, and the endometrium, cervix and prostate gland and in the extracellular matrix of normal liver [28,41,42,50–52].

These findings suggest that an angiogenic environment may precede, rather than result from, the development of neoplasia. Once a tumour becomes established, it may attain characteristics, through further somatic mutations and/or hypoxia [53], which potentiate the angiogenic process and facilitate the invasion and spread of malignant disease.

3. Immune responses and malignant disease

As is the case for angiogenesis there is considerable evidence to suggest that suppression of Th1, often associated with upregulation of Th2 responses, is a common feature of both malignant tumours and of disease processes known to predispose to the development of cancer.

Suppression of local and systemic CMI responses has been confirmed in studies evaluating inflammatory cellular infiltrates of tumours and peripheral blood mononuclear cell responses of patients with malignant disease including non-small cell lung cancer (NSCLC), head and neck, breast, gastrointestinal and genitourinary cancers, melanoma, lymphomas and sarcomas [54–60] and carcinoma *in situ* including Barrett's oesophagus and CIN [55,61,62]. The presence of a dominant Th2 immune response in potentially curable tumours such as lymphomas is associated with a fatal outcome [56]. Delayed hypersensitivity reactions to common T cell recall antigens, a manifestation of CMI, are reduced or absent in patients with a wide range of tumours including gastric cancer, small cell lung cancer, Hodgkin's disease and malignant melanoma [63–67]. In a number of studies, the degree of T cell anergy inversely correlates with patient survival.

More recently, it has been demonstrated that T cells from tumour-bearing animals and humans have abnormalities in their signal transduction pathways which might explain these clinical observations. The T-cell receptor (TCR)-αβ or -γδ chains bind the peptide ligand whilst the associated CD3-γδε and -ζ subunits couple the TCR to intracellular signal transduction components. In *in vivo* tumour models these alterations include a reduction in T-lymphocyte CD3-γ and complete absence in CD3-ζ which is replaced by the Fc εγ-chain. Expression of the tyrosine kinases p56lck and p59fyn is also reduced. There is an associated reduction in the capacity of T-lymphocytes to produce the Th1 cytokines IL-2 and IFN-γ [68–70]. The sequence in which some of these changes in cell signalling occur has recently been elucidated in tumour-bearing nude mice. Alterations in T cell NFkappaB family proteins, with the failure of p65 translocation to the nucleus, occur early and more frequently than the decrease in ζ-chains. The initial changes are followed by the eventual loss of TCR ζ-chain and p56lck and a marked reduction in cytotoxic function [71]. Similar changes in T-lymphocyte signal transduction have been observed in patients with lepromatous leprosy, a condition in which Th2 responses to *Mycobacterium Leprae* predominate [9]. In mice these abnormalities are accompanied by a progressive loss of Th1 populations from the spleen [72], a phenomenon not seen in mice bearing IL-2 secreting tumours [69]. More direct evidence for an association between deficient Th1 responses and a predisposition to cancer comes from gene knockout mice studies. Mice IFN-$\gamma^{-/-}$, IFN-γ receptor$^{-/-}$ or signal transducer and activator of transcription (STAT)$1^{-/-}$ (a component of the IFN signalling pathway) have an increased incidence of tumours [73,74].

A number of processes may be responsible for the T cell anergy seen in malignant disease. Tumours are associated with the overexpression of cyclo-oxygenases which are responsible for the synthesis of prostaglandins, in particular PGE_2, which in turn inhibit Th1 CMI-associated CD4+ lymphocytes whilst stimulating the proliferation of Th2 CD4+ lymphocytes (see later). Recent work has shown that viral agents may produce altered peptide ligands which antagonise binding of the TCR to antigen presenting cells. This results in inhibition of the activation of cytotoxic lymphocytes by blocking CD3-ζ tyrosine phosphorylation [75,76]. Thus, it may be possible for an infection to persist and give rise to the appropriate conditions — angiogenesis and suppressed CMI — which would predispose to the development of malignancy.

The importance of these changes is underlined by studies which have shown the role of the Th1/CMI response in tumour regression and rejection. The role of tumour-specific cytotoxic T cells, a major effector arm of Th1/CMI response, in mediating tumour regression has been established in both animal models [77,78] and in humans [79,80] through studies of adoptive T cell

transfer. However, in the normal setting of progressive tumour growth, the presence of such effector cells is only seen in a small minority of cases. Gene transduction of animal tumour cell lines with both Th1 and Th2 cytokine genes has resulted in cell-mediated responses capable of inhibiting a subsequent challenge of parental tumour cells. However, regression of established tumours typically requires effector cells induced by Th1 cytokines [81] and where tumour rejection has been observed, this has generally been accompanied by the induction of tumour-specific CMI responses. These observations suggest that the growing tumour either fails to stimulate an effective CMI response, or has evaded immune surveillance [82].

A number of experimental and patient studies have evaluated the impact of tumour resection and immunotherapy on immune responses. In patients where complete surgical resection is achieved or where immunotherapy induces either disease stabilisation or an objective tumour response there is a shift in the immune response from a Th2/HI back towards a Th1/CMI dominant profile [8,17,59,60,83–85]. The majority of lymphocytes in malignant pleural effusions of patients with NSCLC are T cells with $<1\%$ natural killer cells and a Th2 phenotype. After IL-2 and IL-12 treatment, the T-helper lymphocytes shift to a Th1 phenotype. IL-2 treatment and TCR-CD3 engagement restores specific antitumour cytotoxicity to these T-lymphocytes. IL-12 synergises with IL-2 in this regard [86,87].

There is increasing evidence that suppression of CMI may precede the development of tumours as indicated by the effects known aetiological agents/diseases for malignancy have on the immune system. Chronic hepatitis B and C predispose to the development of hepatocellular carcinoma [14]. Serological and biochemical studies suggest that acute hepatitis infections are resolved by an effective CMI response, whilst chronic infection is characterised by weak to undetectable CMI responses and/or a dominant Th2 response. The secreted precore antigen (HBeAg), seen in chronic hepatitis B, has been shown to deplete antihepatitis B-specific, Th1 cells which are necessary for viral clearance, whilst enhancing Th2 cytokine-producing cells [12]. In hepatitis C, the activation of Th2 responses plays a role in the development of chronicity [13]. In keeping with this, cultured monocytes from patients with chronic hepatitis C respond to antigen stimulation by increasing the synthesis of the Th2 cytokine IL-10, but not the Th1 cytokine IL-12, when compared with normal controls [11]. Treatment of chronic hepatitis B and C with pro-inflammatory cytokines such as interferon-α and IL-12 results in varying degrees of clearance of viral particles from the liver and clinical remission [88,89]. Treatment, particularly when successful, is associated with a reduction in Th2 responses as determined by serially estimating circulating IL-4, IL-6 and IL-10 levels [88,90].

Human papilloma virus (HPV) infection predisposes to ano-genital malignancy and head and neck cancer and may have a role in the pathogenesis of oesophageal and lung cancer [91]. The principal HPV subtypes associated with neoplastic disease are HPV16 and 18. These produce the E6 and E7 proteins which inactivate the p53 and retinoblastoma (Rb) tumour suppressor proteins, respectively. CMI responses are crucial to the pathogenesis of HPV infection. For example, regression of genital warts is characterised by a localised delayed hypersensitivity response with a pronounced increase in Th1 cells and macrophages. There is secretion of a number of cytokines, with IL-12 being present at very high levels. Furthermore, although cytotoxic T-lymphocyte (CTL) responses have been difficult to detect in patients with HPV infection, CTL responses to E6/E7 proteins are more commonly detectable in women with cervical HPV 16 infection without evidence of CIN than in HPV 16-positive women with CIN [62]. In addition, Al-Saleh and coworkers showed a lower density of IL-2 secreting cells and a higher density of IL-4 positive cells in high-grade squamous epithelial lesions (CIN III) than in the transformation zone of healthy women with biopsies showing squamous metaplasia [92]. These results are supported by studies in women with cervical dysplasia which have shown reduced lymphocyte Th1 immune responses to HPV 16 L1 antigen and E6 and E7 peptides as compared with healthy adults [93], which worsen with the severity of the CIN and in patients with invasive cervical cancer [55].

HIV infection is associated with the development of a variety of tumours, including lymphomas, Kaposi's sarcoma and cervical cancer. The disease is characterised by a progressive decline in CD4+ T-lymphocytes, leading to severe CMI deficiency. A number of studies have shown a skewing of the cytokine response in HIV-infected patients towards the production of Th2 cytokines, with a progressive decline in both Th1 cell numbers and responses [94–96]. Cervical neoplasia develops in approximately 40% of women with HIV infection. A recent study evaluated the cytokine profiles of T-cells in biopsy specimens of normal cervical tissue from HIV-positive women. A shift towards a Th2-immune response compared with normal healthy controls was seen consistent with the overall bias towards Th2 responses in HIV-infected individuals [97]. AIDS-associated Kaposi's sarcoma occurs in up to 20% of HIV-1 infected homosexual men. A gamma herpes virus, HHV8, has been identified in virtually all cases and represents the most likely aetiological agent. Serological studies in immunocompetent individuals with HHV8 infection suggest that HHV8 remains under strict immunological control in healthy HHV8-infected individuals. This supports the contention that HIV-associated CMI immunodeficiency is necessary for HHV8 infection to induce the cell transformation leading to AIDS-associated Kaposi's sarcoma [98].

Extensive inflammatory bowel disease predisposes to colorectal cancer. In this condition Th2/HI responses are dominant with increased serum levels of circulating IL-10 being seen [99]. Likewise obesity, which predisposes to the development of malignant diseases including breast, prostate and colon cancer [100], has been demonstrated to suppress lymphocyte functions, natural killer cell activity and lymphocyte mitogenesis, with reduced T-lymphocyte responses to concanavalin A and B-lymphocyte responses to pokeweed mitogen. Experiments in obese Zucker rats have shown that the suppression of CMI may be reversed by exercise, with restoration of natural killer cell activity and concanavalin A-induced splenic lymphocyte responses [101].

Finally, patients receiving long-term immunosuppressive therapy, for example with agents such as cyclosporin A following organ transplantation, show an increased incidence of both viral and non-virally induced tumours [102,103].

The results of these studies consistently demonstrate that, not only is cancer itself associated with a shift from a Th1 to a Th2 dominant phenotype, but that conditions predisposing to malignant disease induce similar changes. This suggests that in many cases, the immune response shift precedes the development of the neoplastic process and may play a key role in carcinogenesis. As the neoplastic lesion grows it becomes progressively hypoxic. Recent studies indicate that hypoxia is associated with suppression of CMI responses [104,105] which, in turn, would allow escape of the malignant process from immune surveillance and, in conjunction with the induction of angiogenesis, facilitate tumour growth, invasion and metastasis.

4. Angiogenesis and the immune system

The nature of the immune response to any given insult may have a major impact on angiogenesis. Macrophages play an important role in the angiogenic process associated with inflammation, wound healing and tumour growth. The angiogenic potential of interferon-γ Th1-induced and IL-4/glucocorticoid Th2-induced macrophages has been evaluated *in vitro*. These subsets were analysed for a panel of 10 angiogenic growth factors 3 and 6 days after stimulation and compared with unstimulated control macrophages. After 3 days, Th1-stimulated macrophages expressed platelet-derived growth factor (PDGF)-A, midkine (MK), TNF-α, TGF-β1, PDGF-B, hepatocyte growth factor (HGF), TGF-α and insulin-like growth factor (IGF)-I, whilst Th2-stimulated macrophages expressed TGF-β1, PDGF-B, HGF and IGF-1. After 6 days there was considerably enhanced mRNA expression of TGF-α and IGF-I in Th2-activated macrophages as compared with Th1-stimulated macrophages. TNF-α expression was seen in Th1-stimulated cells, but not in Th2-stimulated cells. Coculture experiments revealed that endothelial cell proliferation induced by Th2-stimulated macrophages was 3–3.5 times higher than that induced by Th1-stimulated macrophages. It was postulated that this observation was due to the expression of the predominantly angio-inhibitory cytokine TNF-α by the Th1-stimulated macrophages [106].

A number of recent studies have suggested that, in malignant disease, the inflammatory cell infiltrate contributes significantly to the angiogenic process. In a recent study, we identified foci of PD-ECGF cancer cell positivity in otherwise PD-ECGF-negative tumour samples. The focal positivity was found to correlate with focal neovascularisation and lymphocyte infiltration, suggesting an important role for PD-ECGF in the initiation of the process of angiogenesis in NSCLC which may be contributed to by the inflammatory cell infiltrate [107]. Studies in breast, colorectal, gastric cancer and NSCLC, non-Hodgkin's lymphoma and glioma lend further support to the importance of the inflammatory cell infiltrate, and macrophages in particular, in the induction of angiogenesis in malignant disease [108–113]. In NSCLC, PD-ECGF expression by stromal fibroblasts is associated with increased angiogenesis, in turn associated with intense stromal macrophage infiltration [37]. In colorectal and gastric cancer, and glioma, PD-ECGF expression is seen predominantly in tumour infiltrating macrophages and correlates with increased microvessel counts and, in the gastrointestinal tract tumours, with more advanced primary and metastatic tumours [110–114]. Given the fact that the predominant immune response in solid tumours is Th2 in nature, these findings support the results Kodelja and coworkers discussed above.

A number of cytokines associated with T-cell responses have been shown to influence angiogenesis either directly or indirectly. The Th1 cytokines IL-2, IFN-γ, TFN-α, IL-12 and IL-18, the Th1 associated chemokine IFN-inducible protein (IP)-10 and a number of Th1-cytokine inducing cytokines such as IFN-α and IFN-β have been shown to have anti-angiogenic properties [115–121]. These results suggest that Th1 lymphocyte responses may play a dual role in immune surveillance against tumour development: (a) through activation of effector cells with direct tumoricidal activity; and (b) through the inhibition of angiogenesis. However, it is important to note that classical Th1-associated cytokines may also be pro-angiogenic depending on the background immune response (CMI versus HI) and the cells from which they are produced. For example, in HIV infection-associated Kaposi's sarcoma, a condition in which CMI is suppressed and HI predominant, IFN-γ and TNF-α have been demonstrated to induce angiogenesis [122]. None the less, combinations of IL-12 and TNF-α have synergistic antitumour activity, a finding

postulated as being due, at least in part, to the inhibition of angiogenesis [123].

The angiogenic activity of HI-associated cytokines is a little more complex. IL-6 is a potent angiogenic cytokine which induces the expression of VEGF [124], promotes the growth of endothelial cells immortalised by polyomavirus [124,125] and is overexpressed not only in solid tumours but in a range of inflammatory conditions predisposing to malignant disease including asbestos exposure [31], ulcerative colitis [126], chronic hepatitis [90] and cirrhosis [127]. IL-4 and IL-13 induce marked stimulation of the migration of both cultured bovine and human microvascular cells at a concentration of 0.1 ng/ml [128]. Furthermore IL-4 has been shown to induce the proliferation of, and upregulate vascular cell adhesion molecule (VCAM)-1 and urokinase plasminogen activator expression by, human endothelial cells *in vitro*. IL-4 also stimulates the formation of tube-like structures by bovine and human endothelial cells in type 1 collagen gels and neovascularisation in the rat cornea model [129]. However in the experiments of Volpert and coworkers [128], IL-4 and IL-13 exhibited inhibitory effects on neovascularisation at concentrations tested >0.1 ng/ml. Furthermore, the subcutaneous growth of retrovirally delivered IL-4 transfected into C6 glioma cells has been demonstrated to be reduced, at least in part, through the inhibition of angiogenesis [130]. Finally, the available evidence indicates that IL-10 has predominantly anti-angiogenic activity [131].

In order for an effective CMI response to occur, endothelial cells in an affected tissue need to express endothelial adhesion molecules (EAMs) which facilitate arrest of leucocytes, including neutrophils, monocytes and memory T cells in, and extravasation from, blood vessels. EAMs include E-selectin (CD62E, endothelial leucocyte adhesion molecule-1 or ELAM-1), VCAM-1 (CD106), a member of the immunoglobulin superfamily, and intercellular adhesion molecule-1 (ICAM-1). EAM expression by endothelial cells is controlled by cytokines, with pro-inflammatory cytokines such as IL-1, IL-2, IL-12, IFN-γ and TNF-α upregulating their expression [132,133]. In keeping with this is the observation that IL-2 treatment results in significant increases in soluble E-selectin, ICAM-1 and VCAM-1 blood levels in cancer patients [134]. Recent work suggests that ICAM-1 and ICAM-2 expression are suppressed in malignant cells compared with normal endothelial cells. Unlike the pro-inflammatory cytokines, angiogenic growth factors such as VEGF and bFGF do not appear to upregulate EAM expression. Indeed, bFGF has been demonstrated to downregulate the expression of ICAM-1 on proliferating endothelial cells [135]. Furthermore, the EAM response of tumour-derived endothelial cells to the pro-inflammatory cytokines IL-1α and TNF-α is dampened compared with normal cells [136]. These results suggest that EAM expression is impaired in malignant disease which may, in turn, contribute to the observed downregulation of CMI in these diseases.

There is accumulating evidence that known carcinogens may induce angiogenesis whilst altering the immune system either locally or systemically to provide an oncogenic environment. For example, nicotine, a carcinogen implicated in the pathogenesis of a number of solid tumours, including head and neck, lung, oesophageal, bladder and cervical cancer [137], significantly stimulates endothelial cell DNA synthesis and proliferation at concentrations which are lower than those seen in the circulation after smoking [35]. Exposure of murine T cells to nicotine reduces the percentage of $CD4^+$ T cells expressing the costimulatory counter-receptors CD28 and CTLA-4 and is associated with a reduction in the production of the Th1 cytokines IL-2 and IFN-γ and with upregulation of the Th2 cytokines IL-4 and IL-10 [138]. Similar findings are seen following exposure to ultraviolet light [139–141] and asbestos [31] and in conditions associated with chronic viral infections such as hepatitis B- and C-induced liver cirrhosis (Table 1) [29,30].

Perhaps most provocative of all is the recently published study on the effects of the immunosuppressive agent cyclosporin A on adenocarcinomas of the lung, breast, bladder and kidney [142]. Cyclosporin A induces malignant cell proliferation, invasion and metastasis *in vitro* and/or *in vivo*. Cyclosporin A has been demonstrated to promote the transcription and functional expression of the TGF-β1 gene. TGF-β can promote

Table 1
Angiogenic and immune status of conditions predisposing to neoplastic disease

	Angiogenesis	↓CMI	↑HI
Carcinogens			
Asbestos	+	+	+
Sunlight	+	+	+
Nicotine	+	+	+
Infection			
Hepatitis B & C	+	+	+
HPV	+	+	+
HIV	+	+	+
SV-40	+	?	?
Growth factors and immunosuppressive agents			
IGF-1	+	+	+
TGF-β1 (cyclosporin A)	+	+	+
Chronic inflammatory disease			
IBD	+	+	+
Genetic mutations			
Mutant *TP53*	+	Reduced apoptosis following exposure to Th1 cytokines	

CMI, cell-mediated immunity; HI, humoral immune response; HPV, human papilloma virus; HIV, human immunodeficiency virus; SV-40, simian virus 40; IGF-1, insulin-like growth factor 1; TGF-β1, transforming growth factor-beta 1; IBD, inflammatory bowel disease.

Table 2
Angiogenic and immune status of pre-malignant diseases

	Angiogenesis	↓CMI	↑HI
Lung			
Metaplasia	+	?	?
Dysplasia	+	?	?
CIN	+	?	?
Cervix			
Metaplasia	+	?	?
Dysplasia	+	+	+
CIN	+	+	+
Breast ductal carcinoma *in situ*	+	?	?
Barrett's oesophagus	?	+	?
Colorectal adenoma	+	↑ COX − 2+	

CIN, cervical intraepithelial neoplasia.

tumour cell proliferation, invasion and metastasis. Antibodies to TGF-β block the observed effects of cyclosporin A on the cell lines tested [102,142]. TGF-β1 is also a potent inhibitor of Th1/CMI immune responses [143,144] and has been shown to direct the immune response towards a Th2/HI phenotype [145]. Although the results are conflicting there is considerable evidence that, under the appropriate circumstances, TGF-β1 is angiogenic [146]. TGF-β1 significantly induces the synthesis of VEGF by cancer cells [147]. As discussed earlier, tumour-infiltrating macrophages have been implicated as playing a key role in tumour-associated angiogenesis. TGF-β is chemotactic for, and induces the production of growth factors and proteases by, macrophages. These include IL-6 and urokinase, the latter playing an important role in the degradation of the extracellular matrix, a process required for angiogenesis [31,148,149]. Therefore, cyclosporin A not only induces suppression of CMI but, through the induction of the cytokine TGF-β1, may enhance tumour cell proliferation, further suppressing CMI, upregulating HI and, finally, inducing angiogenesis.

Collectively, the findings indicate that induction of angiogenesis and suppression of CMI, often associated with upregulation of Th2 immune responses, are common features of cancer, irrespective of the site or histological subtype of the malignant disease. Furthermore, these processes appear to precede and play a significant role in carcinogenesis (Table 2).

5. Molecular models linking angiogenesis and T-helper responses: potential novel targets for chemoprevention and treatment of malignant disease

5.1. Cyclo-oxygenases-1 and -2 (COX-1 and -2)

COX-1 and COX-2 catalyse the first two steps in prostanoid synthesis. COX-1 activity is constitutively expressed in nearly all cell types and plays a central role in many normal physiological processes, such as cytoprotection of gastric mucosal surfaces, through synthesis of prostacyclin and PGI_2. COX-2 is rarely expressed in normal tissues but is induced in response to inflammatory stimuli. These stimuli include the pro-inflammatory cytokines, IL-1β, IL-2, IFN-γ and TNF-α which in turn are induced by the appropriate presentation of antigens, including viral and bacterial particles such as bacterial lipopolysaccharide (LPS). The precise prostaglandin synthesised as a result of COX-2 induction depends on the specific synthase enzyme(s) present in the cell [150–152].

Prostaglandin E_2 (PGE_2) is synthesised by prostaglandin E_2 synthase and may have a role in the pathogenesis of malignant disease. On binding to its receptor, PGE_2 induces intracellular cyclic adenosine monophosphate (cAMP) accumulation and, as a result, through the activation of the protein kinase A(PKA) signal transduction pathway, may stimulate cell growth, alter cell adhesion and inhibit apoptosis. In peripheral blood mononuclear cells, cAMP accumulation results in the inhibition of the proliferation of Th1 cytokine-producing cells with the opposite effect on Th2 responses [153]. Recent work has clearly shown that PGE_2 inhibits the production of the Th1 cytokines IL-2, IFN-γ and TNF-α by T-lymphocytes whilst upregulating the HI-associated cytokine IL-6 [154]. Exposure to ultraviolet (UV) irradiation is associated with suppression of systemic CMI despite the fact that all energy contained by the UV wavelengths is absorbed by the epidermis and upper layers of the dermis. Recent work has demonstrated that keratinocytes may produce IL-10 on exposure to UV light. Furthermore there is evidence of the systemic production of IL-4 induced by PGE_2 synthesis in keratinocytes [155].

Overexpression of COX-2 and/or PGE_2 is seen in a range of malignant diseases including those arising from the head and neck, breast, lung, stomach, colon and rectum. In NSCLC, tumour cell-derived soluble mediators and PGE_2 have been demonstrated to enhance transcription and protein production of the Th2 cytokine IL-10 from peripheral blood lymphocytes. The presence of specific anti-IL-10 antibodies results in enhanced synthesis of IFN-γ, the Th1 cytokine, from these cells. The shift to a Th2-dominant response in malignant disease results in the downregulation and inhibition of CMI-mediated antitumour immune responses [150–153,156–158].

In recent work, COX-2 overexpressing colorectal cancer cells have been found to synthesise a number of growth factors which may contribute to angiogenesis including VEGF, bFGF, bFGF-binding protein, TGF-β, PDGF-B, endothelin-1 and inducible nitric oxide synthase (iNOS) and to stimulate both endothelial migration and tube formation. The effect is inhibited by antibodies to combinations of the angiogenic factors, by a selective COX-2 inhibitor and by aspirin. COX-2

inhibition has no effect on the synthesis of angiogenic factors or on angiogenesis itself in COX-2-negative cells. However, treatment of endothelial cells with either aspirin or COX-1 antisense oligonucleotide inhibits angiogenesis. Therefore, cyclo-oxygenases induce angiogenesis by at least two mechanisms; COX-2-enhanced production of angiogenic growth factors in COX-2-positive cancer cells, and COX-1 regulation of angiogenesis in endothelial cells [39]. In keeping with these observations, PGE_2 is itself an angiogenic growth factor which induces the synthesis of angiogenic growth factors such as VEGF [159–161]. Furthermore, upregulation of COX-2 is associated with activation of matrix metalloproteinase-2 and an increase in the expression of membrane-type metalloproteinases which both facilitate tumour invasion and angiogenesis [162].

By-products of the metabolism of arachidonic acid, the precursor compound for prostaglandin synthesis, by cyclo-oxygenases include a number of known mutagens including malondialdehyde, itself a highly reactive compound capable of forming adducts with DNA which ultimately may lead to DNA damage and carcinogenesis [150,151,163,164].

Therefore, induction of cyclo-oxygenases, in particular COX-2, may result in: (a) the induction of angiogenesis through the associated upregulation of angiogenic growth factors; and (b) a shift in immune responses from a Th1/CMI to a Th2/HI dominant phenotype. This would facilitate the escape of established neoplastic lesions from immune surveillance and enhance their growth and metastatic potential. The evidence also indicates that chronic induction of COX-2 precedes the development and, through the local production of carcinogens such as malondialdehyde, may participate in the neoplastic process itself.

5.2. Insulin-like growth factor-I (IGF-I)

The insulin-like growth factor (IGF) family comprises three ligands, three receptors and six binding proteins. Many normal cells require IGF-I for optimal growth *in vitro* including epithelial cells, human diploid fibroblasts, smooth muscle cells, chondrocytes, osteoblasts, T-lymphocytes, myeloid cells and bone marrow stem cells. *In vitro* and *in vivo* studies indicate that IGF-I is a cell survival, mitogenic and transforming factor which is involved in the pathogenesis and growth of malignant tumours. Indeed, IGF-I acts as an autocrine growth factor for many tumours including lung and breast cancer [165–167]. IGF-I inhibition of apoptosis has been associated, in various circumstances, with (a) inhibition of the downregulation of Bcl-2, (b) upregulation of Bcl-2 and Bcl_xL and (c) inhibition of the upregulation of caspases which may occur in physiological stress situations such as hypoxia and withdrawal of growth factors [168–170].

IGF-I acts predominantly through the IGF-I receptor (IGF-IR) which, in keeping with the central role of this peptide in normal growth and development, is constitutively expressed in most cells in the body. The IGF-IR is a tyrosine kinase receptor. The central role of IGF-IR in the transformation of many cell types is best illustrated by the effects of disruption of the IGF-IR signal transduction pathway. This reverses the transformed phenotype and/or inhibits tumorigenesis and/or induces loss of metastatic potential in human breast, lung and ovarian cancer and melanoma tumour models amongst others [167].

The activated IGF-IR induces tyrosine phosphorylation of insulin receptor substrate (IRS)-1, which provides binding sites for proteins with the appropriate Src homology two domains including the 85 kDa regulatory subunit of phosphatidylinositol (PI) 3′-kinase. IRS-1 was originally described as a docking protein specific for insulin and IGF-I receptors. Recent work has indicated that IRS-1 is at the crossroads of several signalling pathways, including those for both Th1 cytokines such as the IFNs and the Th2 cytokines such as IL-4, where it interacts with the Janus kinases (JAKs) [171–173]. Cytokines such as the IFNs cause a decrease in IGF-I and IGF-IR transcription and appear, as has been documented for IL-1, to play an important role, at least as cofactors, in the induction of an IGF-I non-responsive state in chondrocytes during experimentally induced arthritis [174–176]. In hepatocytes the pro-inflammatory, CMI-associated cytokines IL-1β and TNF-α inhibit growth hormone-induced IGF-1 mRNA synthesis [177]. Likewise, IL-1 has been implicated in the decreased IGF-I levels seen in sepsis [178]. In contrast, the HI cytokine IL-6 enhances the expression of IGF-I in hepatocytes and osteoblasts, an effect which, in the latter case, may lead to a secondary increase in bone formation [177,179].

IGF-I has recently been shown to potentiate IL-1β-induced COX-2 and iNOS expression in glomerular mesangial cells. This, in turn, leads to enhanced PGE_2 and nitric oxide synthesis. IGF-I enhances IL-1β-induced p38 mitogen-activated protein kinase (MAPK) phosphorylation and SAPK activation, suggesting an important role for this signalling pathway in the upregulation of COX-2 and iNOS observed [180]. The enhanced production of PGE_2 under these circumstances may lead to the downregulation of local, and indeed, systemic Th1 responses and upregulation of Th2 responses as described earlier. In osteoblasts, PGE_2 has been demonstrated to enhance IGF-I synthesis through a cAMP-dependent protein kinase A pathway indicating the presence of a positive feedback loop [181].

Although IGF-I is a lympho- and myeloproliferative factor and has a generalised potentiating effect on immune responses, a number of studies suggest that, under the appropriate circumstances, the growth factor

may block the upregulation of CMI responses to antigens and injury. For example, renal ischaemic injury is associated with increased cytokine and major histocompatibility complex (MHC) expression and a mild interstitial infiltrate in the affected kidney. IGF-I therapy blocks this pro-inflammatory response inhibiting the expression of TNF-α [182]. Likewise, treatment with IGF-I has been shown to downregulate TNF-α serum levels in an *in vivo* sepsis model as compared with untreated controls [183]. Furthermore, overexpression of IGF-IR protects cells against apoptosis from Th1/CMI-associated cytokines such as TNF [184]. IGF-I augments HIV-1 replication in phytohaemaglutinin-stimulated PBMCs *in vitro* [185] and induces B-lymphocyte proliferation and immunoglobulin synthesis including IgE production, findings consistent with a HI response. In the latter case, B-lymphocyte class switching was found to occur independently from an IL-4- or IL-13-dependent mechanism [186]. In keeping with these observations, IGF-I depletion in sham operated rats is associated with impaired wound healing and a 50% reduction in wound macrophages as compared with normal controls. This demonstrates a pivotol role for IGF-I in normal wound healing, a process associated with suppression of CMI immune responses [187].

IGF-I is an angiogenic growth factor which is required for the optimal growth of endothelial cells *in vitro*. In keeping with the enhancement of COX-2 activity, IGF-I-induced angiogenesis is associated with the synthesis of angiogenic growth factors such as VEGF [188]. Furthermore, there is considerable evidence that IGF-I may itself be upregulated by other angiogenic growth factors. As has been mentioned earlier, IL-6 and PGE_2, themselves angiogenic agents, have been shown to induce IGF-I synthesis. Apart from IL-6 and PGE_2, other angiogenic growth factors shown to upregulate the IGF-I/IGF-IR system include PDGF [189].

Recent work has suggested that antisense IGF-I/IGF-IR therapy may have a role to play in the treatment of malignant disease. Antisense therapy results in reduced tumour cell growth *in vitro* and in the inhibition of tumorigenicity and induction of tumour regression *in vivo* [167,190–192]. When IGF-I synthesis in transfected cells is inhibited, the expression of MHC class I antigen is increased at least 4-fold. This is associated with a tumour-specific immune response [191]. Indeed, animals treated with IGF-IR antisense cells may become resistant to subsequent challenge with wild-type cancer cells. This has been observed to occur even if the initial inoculating cells were from different tumours or from unrelated animals. The reasons for this are unclear and require further evaluation [167].

These results indicate an important role for growth factors such as IGF-I in the close inter-relationship between angiogenesis and the immune response in normal physiological processes and disease. The evidence suggests that angiogenic, HI-dominant immune responses induce growth whilst anti-angiogenic, CMI-dominant responses inhibit tissue proliferation. The contribution of the cell growth factor IGF-I to angiogenic and anti-inflammatory processes may explain the increased incidence of solid tumours such as breast, prostate and colorectal cancer seen in acromegalic patients and those with high normal IGF-I serum levels [193–195].

5.3. Inactivation of p53

TP53, a tumour suppressor gene, plays a central role in the regulation of cell proliferation, causing arrest of the cell cycle in the G1 phase, facilitating DNA repair and decreasing DNA synthesis. Under the appropriate conditions, such as significant growth factor deprivation, hypoxia and irradiation, p53 is upregulated and induces apoptosis. Current evidence indicates that inactivation of p53 protein or the *TP53* gene, or mutation of both *TP53* alleles plays an important role in oncogenesis. Viral infections known to inactivate p53 are associated with an increased risk of malignant disease. These include the HPV viruses, in particular, the 16 and 18 serotypes [62] and Simian virus-40 (SV-40), a virus recently implicated in the pathogenesis of a range of tumours including mesothelioma, ependymomas, choroid plexus tumours, bone tumours and sarcomas. Like HPV, SV-40 virus produces an oncoprotein, SV-40 large T antigen, which binds p53 and each of the retinoblastoma family proteins, pRb, p107, and pRb2/p130 [196,197]. The importance of the inactivation of p53 in the malignant transformation of cells is underlined by the experiments undertaken in the successful creation of human tumour cells from normal epithelial cells and fibroblasts. The investigators included this specific step as one of the defined alterations made to the cells [198]. Inherited abnormalities of the *TP53* gene, as seen in patients with Li Fraumani syndrome, also predispose to the development of malignant disease. In cultured fibroblasts from patients bearing the Li Fraumeni genotype, the subsequent loss of the wild-type allele is associated with loss of expression of thrombospondin-1, an endogenous anti-angiogenic agent. This results in the fibroblasts taking on an angiogenesis-inducing phenotype, a process which precedes the development of the malignant phenotype [199,200].

Not only is loss of wild-type p53 associated with the development of malignant disease but mutant p53 may actually confer a growth advantage to malignant cells and inhibit p53-independent apoptotic pathways [201,202]. This may in part be explained by the role the p53 protein plays in the regulation of growth factor pathways including that activated by IGF-I. Under normal conditions, IGF-I upregulates p53 expression [203–205]. p53 subsequently represses the transcription

of IGF-IR [206,207] and induces the expression of IGF-binding protein 3, a factor which antagonises IGF-I activity and is capable in its own right of inducing apoptosis [208,209]. In contrast, some mutations in the p53 protein appear to have the capacity to derepress the IGF-IR promoter leading to growth enhancement by locally produced or systemic IGF-I [210]. Apart from playing a role in upregulating cell growth and growth factor receptors, mutations in *TP53* may potentiate protein kinase C induction of VEGF synthesis which may result in increased angiogenesis [211].

Growth factors may also antagonise apoptosis through suppression of p53 expression. Granulocyte macrophage-colony stimulating factor (GM-CSF) is a growth factor for acute myeloblastic leukaemia cells. In keeping with this, GM-CSF upregulates mdm-2, a protein which binds to and inhibits wild-type p53, while GM-CSF deprivation is associated with upregulation of p53 and apoptosis [212]. γ-irradiation p53-dependent apoptosis may be antagonised by specific growth factors such as IL-3 and erythropoietin, a process which appears to be due to activation of Jak kinase-dependent signalling. This observation suggests a functional link between activation of certain Jak kinases and suppression of p53-mediated cell death. Signal targets of the Jak kinase pathways include the Bcl-2 family proteins. Indeed, upregulation of Bcl-2 and Bcl-xL are as effective as IL-3 and erythropoietin in blocking irradiation-induced apoptosis as described earlier for IGF-I [213]. In keeping with the observations for GM-CSF, withdrawal of IL-3 from IL-3 growth-dependent cells results in upregulation of p53. Furthermore, there is associated downregulation of the IGF-IR [214]. p53 upregulation in these circumstances facilitates retinoblastoma protein cleavage in IL-3-deprived cells indicating a new pro-apoptotic activity for the tumour suppressor protein [215].

Cytokines which induce apoptosis do so predominantly through the activation of caspases which link specific cytokine receptors to p53 [215,216]. As has been mentioned earlier the Th1 cytokine IFN-γ plays an important role in immune surveillance in immunocompetent individuals. Acting through STAT1, IFN-γ-induced apoptosis is associated with upregulation of p53 and p21/WAF1/CIP1 [217]. In keeping with this, IFN-γ insensitive mice have an increased incidence of tumours. This is exacerbated in *TP53*$^{-/-}$ mice. Tumours not only develop more rapidly but a broader spectrum of malignant disease is seen [74]. TNF-α also induces apoptosis through both p53-dependent and p53-independent pathways [218]. In neoplastic cells expressing normal and mutant alleles, such as the C6 rat glioma cell line, wild-type p53 expression is enhanced and mutant p53 suppressed by TNF-α-induced apoptosis indicating that the cytokine may activate the function of wild-type p53 through the suppression of mutant p53 [219]. Recent work has clearly shown that there is significant cross-talk between the IFN and TNF-α pathways. This allows the cell proliferation suppressor dsRNA-dependent protein kinase (PKR) to be recruited from the IFN pathway. PKR appears to have an important role in the TNF-α pathway, its upregulation preceding the induction of wild-type p53 expression [220].

Studies in the gastric cancer cell line SNU-16 and normal fibroblasts indicate that deregulation of c-Myc sensitises cells to the apoptotic effects of p53 induced by TNF-α [221,222]. TNF-α-induced apoptosis in ME-180 cells is not only associated with upregulation of wild-type p53 but with the stabilisation of the p21/WAF1/CIP1 protein. Subsequent proteolysis of p21/WAF1/CIP1 occurs before the onset of DNA fragmentation. The kinetics of p21/WAF1/CIP1 proteolysis closely parallel those of poly(ADP-ribose)polymerase, suggesting cleavage of p21/WAF1/CIP1 by activation of an apoptotic protease. This indicates the early onset of genotoxic stress in cells committed to TNF-mediated apoptosis. TNF-induced p21/WAF1/CIP1 proteolysis may contribute to the apoptotic process by disrupting p53 signalling, altering cell cycle inhibition and limiting cellular recovery from genotoxic stress [223]. The death receptors DR4 and DR5 are members of the TNF receptor family. DR4 and DR5 bind the ligand TRAIL and thereby engage the caspase cascade to induce apoptosis. Both are upregulated by TNF-α through p53-dependent and p53-independent mechanisms. In the latter case, TNF may act as a gene transcription factor. Furthermore, as for other p53-regulated genes induction of DR5 by ionising irradiation appears to occur only in wild-type p53-expressing cells [218]. While the CMI-associated IFNs activate STAT1, the HI-associated cytokine IL-6 signals through STAT3, which in turn upregulates p21/WAF1/CIP1 expression. In human osteoblastic cells, this upregulation of the cell cycle regulatory protein appears critical to the pro-differentiating, anti-apoptotic effects of IL-6, which is in contrast to the situation described for Th1-associated cytokines [224].

Through the detection of anti-p53 antibodies the presence of a HI response to mutant p53 has been detected in a significant proportion of patients with malignant tumours, including those of the central nervous system and lung cancer [225,226]. More recently, it has been established that HLA class II restricted CD4+ T cells reactive to p53 proteins do exist in healthy individuals and it has been suggested that the epitopes are probably ignored by the immune system under physiological conditions [227].

In summary, loss of wild-type p53 impairs Th1 cytokine-induced apoptosis, facilitates angiogenesis and, in many circumstances, appears to lead to the upregulation of growth factors and their receptors which may lead to or augment cell transformation, the establish-

ment of a neoplastic lesion and subsequent dissemination of the malignant disease process.

6. Chemoprevention studies

Recent work has demonstrated that long-term ingestion of aspirin protects against the development of cancer, in particular colorectal disease where the incidence is reduced by as much as 45% and oesophageal cancer [228–230]. Furthermore, natural and synthetic non-specific COX-1 and -2 and specific COX-2 antagonists have been shown to inhibit carcinogenesis in lung [231], breast [232], oral [233], gastric, duodenal and colon [234,235] cancer while sulindac causes a reduction in the number of polyps in patients with familial polyposis coli [236].

Aspirin, an inhibitor of COX-1 and COX-2 activity, is an anti-angiogenic agent [39]. Curcumin, a naturally occurring dietary polyphenolic phytochemical isolated from the commonly used spice turmeric, is also an inhibitor of COX-2 [235] with anti-angiogenic properties [237]. Furthermore, non-steroidal anti-inflammatory agents including aspirin, sulindac and indomethacin, have been shown to attenuate tumour-mediated immune suppression [150–152,231,238–240].

A number of cytokines involved in CMI responses have anti-angiogenic properties. These include IL-2, IL-12, IL-18, the IFNs, IP-10 and TNF-α [115–121]. These properties would suggest chemopreventive roles for Th1 cytokines. Recent studies support this contention. For example, interferon-α reduces the risk of developing hepatocellular cancer in patients with hepatitis B- and C-induced cirrhosis of the liver [14].

Recent clinical studies indicate that anti-oestrogens such as tamoxifen and raloxifene may have a role to play in the chemoprevention of breast cancer with the results of further ongoing trials keenly awaited [241,242]. Experimental evidence indicates that the chemopreventive activity may be related, at least in part, to the anti-angiogenic effects of these agents which has been demonstrated in both the chorioallantoic membrane assay and in treated MCF-7 breast cancer xenografts [243,244].

Anti-angiogenic agents without Th1 induction (or indeed with Th1 suppression activity) also appear to protect against the development of malignant disease. Treatment of hypertensive patients with captopril, an angiotensin-I-converting enzyme (ACE) inhibitor, is associated with a reduced risk of developing malignant disease, in particular smoking-related tumours such as lung cancer and female specific malignancies [245]. As well as acting directly on capillary cells, inhibiting their chemotaxis, captopril is a free sulphydryl donor which, in combination with plasminogen activators, generates angiostatin, a potent anti-angiogenic compound, from plasminogen [246]. Captopril also inhibits zinc-dependent endothelial cell gelatinase matrix metalloproteinase activity. Gelatinases play an important role in the degradation of blood vessel basement membranes and the extracellular matrix, key processes necessary for angiogenesis [247]. However, ACE inhibitors suppress production of interferon-γ and IL-12 by PBMC stimulated with LPS or SAC suggesting an anti-Th1 affect [248].

These observations are in keeping with the contention that both angiogenesis and suppressed CMI are required for the development of neoplastic disease, a normal Th1 response negating the potential pro-oncogenic environment created by local or generalised angiogenesis and vice versa.

7. Hygiene, immunisation and cancer

Western countries have seen a significant rise in the incidence of many malignant diseases over the past few decades. This has affected all age groups with particularly significant annual rises being observed in germ cell tumours, lymphoma and melanoma [137,249,250]. The increase in malignant disease has occurred on a background of an increased incidence of other conditions characterised by a predominant Th2 immune response including the atopic conditions allergic rhinitis, eczema and asthma [251]. Indeed, the presence of malaria-specific IgE, a Th2 response, has recently been reported as a risk factor not only for atopy but also for malignant disease [252].

Better housing and hygiene, and a decline in a variety of infectious diseases combined with, and in part due to, current immunisation regimens may contribute to these phenomena through their effects on the development and maturation of the immune system in early life. The fetal immune system develops within an intrauterine environment which is predominantly Th2 in nature [3]. At birth, cord blood lymphocytes are capable of responding to mitogenic and antigenic stimuli but there is a general skewing of the T cell immune response towards the production of Th2 cytokines [253,254]. Development of Th1 responses are delayed and driven largely by exposure to micro-organisms in postnatal life. Frequent infection in early infancy causes induction of Th1/CMI-associated cytokines such as IFN-γ, IL-12 and IL-18 which in turn would lead to suppression of Th2 responses and unfavourable circumstances for allergen sensitisation [255,256].

Recent studies indicate that immune responses to specific infectious agents may have a more generalised, long-term effect on the immune system. Early life exposure to measles and Bacille Calmette-Guerin (BCG), which both induce predominantly Th1 responses, is associated with a reduced incidence of subsequent

atopia [257,258]. However, whilst infections such as measles, mumps and rubella tend to induce Th1 immune responses, the currently used vaccines against these agents induce Th2 responses leading to the production of antibodies which protect against subsequent infections. The Th2 immune response generated may be as a result of the vaccine antigens and, in particular, the adjuvant employed [259,260]. Furthermore the pattern of biased Th2 responses to vaccine antigens persists after booster immunisation in later life [261]. The data support the concept of an 'immune memory', the immune responsiveness of the individual being dictated to a greater or lesser extent by the specific immune stimulants to which he or she is exposed in infancy [8,261].

As such, lifestyle changes in the Western world would predispose the population to respond to novel antigenic stimuli with Th2-dominant, Th1-suppressed immune responses. This change, when associated with the induction of angiogenesis, may contribute to, to a greater or lesser extent, the increased incidence of malignant disease seen in Western world countries.

8. Conclusions

Prolonged exposure to known carcinogenic infective, chemical or physical agents and growth factors, or inhibiton of the function of tumour suppressor genes, may predispose the individual to the development of a local and/or systemic environment in which angiogenesis is upregulated, Th1/CMI responses are suppressed and Th2/HI responses predominate. The likelihood of such an environment occurring is potentiated by a Western lifestyle in which: (a) the development of Th2/HI, rather than Th1/CMI responses to novel antigens may predominate due to increased hygiene and lack of exposure to infective agents in early life; (b) exposure to carcinogens is increased; and (c) obesity and lack of exercise are commonplace.

The angiogenic process would ensure the presence of a nutrient-rich environment in the affected tissue or tissues. Under these circumstances the likelihood of significant somatic mutations occurring, or of inherited genetic mutations becoming penetrant, would be enhanced. A developing neoplastic cell and, subsequently, tumour would be protected from: (a) immune surveillance by the local and systemic suppression of Th1 responses; and (b) from apoptosis by the presence of high local survival growth factor levels. The importance a pre-existing vasculature bed may play in the pathogenesis of malignant disease has recently been demonstrated in elegant *in vivo* experiments which show that at least some tumours grow initially by co-opting existing host vessels [263]. As the tumour develops it would outgrow the existing blood supply and become hypoxic with a resultant increase in angiogenesis and further suppression of CMI. At the same time the tumour may acquire further genetic mutations facilitating invasion of local tissues and metastases.

This sequence of events is supported by the observation that inhibitors of angiogenesis and factors which upregulate Th1 immune responses protect against the development of malignant disease in animal models and patients. Furthermore the argument is consistent with the theory that tumorigenesis may start in cells with a normal mutation rate and that selection is the major mechanism that drives somatic evolution towards cancer development [262].

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