THE LINK BETWEEN INFLAMMATION AND CANCER

Wounds that do not heal
Cancer Treatment and Research
Steven T. Rosen, M.D., Series Editor

THE LINK BETWEEN INFLAMMATION AND CANCER

Wounds that do not heal

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THE LINK BETWEEN INFLAMMATION AND CANCER

Wounds that do not heal
CONTENTS

Foreword vii
Angus G. Dalgleish and Burkhard Haefner

Contributors xi

1. Inflammation and Cancer: The role of the immune response and angiogenesis 1
Angus G. Dalgleish and Ken O’Byrne

2. Chronic Inflammation and Pathogenesis of GI and Pancreatic Cancers 39
Lindsey N. Jackson and B. Mark Evers

3. Cytokines, NF-κB, Microenvironment, Intestinal Inflammation and Cancer 67
Arndt J. Schottelius and Harald Dinter

4. Regulation of NF-κB Transcriptional Activity 89
Linda Vermeulen, Wim Vanden Berghe and Guy Haegeman

5. The Role of Immune Cells in the Tumor Microenvironment 103
Theresa L. Whiteside

Isaac P. Witz
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.</td>
<td>CD95L/FasL and Trail in Tumour Surveillance and Cancer Therapy</td>
<td>141</td>
</tr>
<tr>
<td></td>
<td>Harald Wajant</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Infection &amp; Neoplastic Growth 101: The required reading for microbial pathogens aspiring to cause cancer</td>
<td>167</td>
</tr>
<tr>
<td></td>
<td>Jessica Bertout and Andrei Thomas-Tikhonenko</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Cytokines as Mediators and Targets for Cancer Cachexia</td>
<td>199</td>
</tr>
<tr>
<td></td>
<td>Josep M. Argilés, Sílvia Busquets and Francisco J. López-Soriano</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>Targeting NF-κB in Anticancer Adjunctive Chemotherapy</td>
<td>219</td>
</tr>
<tr>
<td></td>
<td>Burkhard Haefner</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Index</td>
<td>247</td>
</tr>
</tbody>
</table>
A link between inflammation and cancer has been established many years ago, yet it is only recently that the potential significance of this connection has become apparent. Although several examples of chronic inflammatory conditions, often induced by persistent irritation and/or infection, developing into cancer have been known for some time, there has been a notable resistance to contemplate the possibility that this association may apply in a causative way to other cancers. Examples for such progression from chronic inflammation to cancer are colon carcinoma developing with increased frequency in patients with ulcerative colitis, and the increased incidence of bladder cancer in patients suffering from chronic Schistosoma infection. Inflammation and cancer have been recognized to be linked in another context for many years, i.e., with regards to pathologies resembling chronic lacerations or ‘wounds that do not heal.’ More recently, the immunology of wound healing has given us clues as to the mechanistic link between inflammation and cancer, in as much as wounds and chronic inflammation turn off local cell-mediated immune responses and switch on growth factor release as well the growth of new blood vessels – angiogenesis. Both of these are features of most types of tumours, which suggest that tumours may require an immunologically shielded milieu and a growth factor-rich environment.

The discovery that some cancers are associated with viral infections and that these viruses only cause cancer in a small percentage of infected individuals has served to highlight that these ‘oncogenic’ viruses are extremely ‘mild’ with regard to the time it takes to induce cancer and that other factors must be involved. Examples of these viruses include human papilloma viruses (HPV), Epstein Barr Virus (EBV) and the two hepatitis viruses, HBV and HCV. HPV causes cervical cancer in only a small percentage of infected women, most of whom eventually become clear of infection. Chronic infection with HPV would appear to be more likely in the presence of additional infectious agents leading to established infection and chronic cervicitis. EBV only rarely causes cancer in
Western populations, but where it is clearly linked with cancers in a causative sense, e.g. Burkitt’s Lymphoma (BL) in Africa and nasopharyngeal carcinoma (NPC) in China, there are other chronic co-factors involved. In the case of BL, there is a strong link to malaria and with NPC, salted fish acting as an irritant/carcinogen is required. However, HBV (and HCV) infection, over decades, will induce chronic hepatitis which evolves into cirrhotic changes from which hepatomas develop. Although co-factors may be involved, they do not appear necessary. These examples emphasize that these are extremely weak oncogenic viruses contained for years by an effective immune response (the only clear link between EBV and tumours (lymphomas) in the West are seen in immunosuppressed patients and the tumours often resolve upon reversal of immunosuppression). The bottom line is that all these viruses usually only cause malignant transformation after many years of inflammatory infection.

The rapid growth in our understanding of molecular signaling pathways in the past decades has taught us that some of these regulatory cascades appear to be key to the development of cancer from chronic inflammatory conditions. Pivotal among these signaling circuits appears to be the NF-κB pathway, crucial for the regulation of immune and inflammatory responses, and linked to non-inflammatory core pathways in oncogenesis, such as the p53 pathway. A major focus of this book, therefore, is the NF-κB pathway as well as the interaction between the tumour and its microenvironment involving the immune response, apoptotic pathways, cytokines and the selectins. In this volume we have tried to highlight the complexity of these processes, yet at the same time show just how fundamental the basic link is. Inevitably this means looking at the same scene from different angles, and hence, there is some degree of overlap. However, the detail is so impressive that we feel this helps enforce the clarity of the overall picture, which only goes to raise the question: ‘Why has the link been denied for so long?’ The major impact of this book, however, should be to emphasize the obvious dramatic therapeutic and preventative implications. Chronic viral infections, for example, can be vaccinated against. For instance, the widespread availability of a HBV vaccine for several decades has greatly reduced the incidence of hepatoma and, as such, can claim to be the first effective preventative cancer vaccine. The common ‘non-infection’-related tumours such as colon, lung, breast, and prostate cancer, may be ‘preventable’ by regular administration of anti-inflammatories such as
aspirin. More specific drugs may be derived from studies included in this book which will lead to more effective preventative strategies as well as treatments. The link between an inflammatory environment and more rapid progression has been recognized for a number of tumours, including breast cancer, clearly suggesting that anti-inflammatory agents may have a major role in therapy. Unfortunately, several studies using the new COX-2 inhibitors have had to be halted because of the association with increased heart attack risk. Aspirin of course is a COX inhibitor which protects against heart attacks and is an obvious candidate. Unfortunately, gastritis and increased gastric bleeding tendency have reduced enthusiasm for such studies even though aspirin has been reported to be preventative for a number of solid tumours, including breast cancer as well as colorectal cancer. Clearly, there still is plenty of scope for the development of improved anti-inflammatory drugs which can be used in cancer prevention as well as treatment.

The recognition of the link with chronic inflammation allows for new ways of understanding how cancers progress and how different types of cancer can show similar responsiveness to drug treatment, which is already being seen for Avastin, a monoclonal antibody which targets vascular endothelial growth factor and is active in disparate tumour types. Another example of how this approach can influence cancer treatment is Revlimid, a Thalidomide analogue developed by Celgene. It was selected for its anti-TNF and thus anti-inflammatory activity but was found to also have strong anti-angiogenic properties and to stimulate the cell-mediated immune response. This compound may thus deliver an ideal three-way blow to tumours and has recently been shown to be highly active against multiple myeloma. It would be surprising if it did not act against other tumour types as well. Thus, we feel confident in proclaiming that this book does not merely cover a speculative theory, but rather the basis for a therapeutical revolution in the treatment of cancer.

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Chapter 1

INFLAMMATION AND CANCER:

The role of the immune response and angiogenesis

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Abstract: The link with chronic inflammation and cancer has been recognized for certain cancers for several decades. However, only recently has the biology of chronic inflammation begun to be understood, to the point that it may play a major role in tumour development. The biology of chronic inflammation has many similarities with that of wound healing. In particular, local cell mediated immunity is attenuated and angiogenesis is increased along with other growth factors. When present long term, this provides the ideal environment for mutated cells to be nurtured and escape immune surveillance. It is of note that this process still appears to take two or three decades, as witnessed by the close association between chronic ulcerative colitis and colon cancer as well as chronic hepatitis and hepatocellular carcinoma. Closer study of the inflammatory pathways show the close interaction with apoptosis and anti-apoptotic pathways, as well as the main tumour suppressor genes, such as p53, as well as a number of growth factors, such as the insulin-like growth factor. A full study of these processes reveals that there are key molecules in these pathways which may provide therapeutic as well as anti-inflammatory targets.
Chapter 1

1. INTRODUCTION

The association between chronic inflammation and cancer is not new. However, the previously recognized associations had limited practical implications and it is only the relatively recent understanding of molecular mechanisms of cancer and the interaction of the immune system and angiogenesis that have led to the potential for targeted intervention to both prevent and treat a number of different cancers.

It was recognized in the nineteenth century that certain cancers were due to chronic irritation, notably the scrotal cancer of chimney sweeps where the irritant was the coal dust and male breast cancer associated with chronic irritation by braces. It was Virchow in 1863 who wrote about the possibilities of cancers arising from sites of chronic inflammation, noting that cancers are similar to wounds that failed to heal. The striking similarities between wound healing and tumour stroma have been reviewed more recently by Dvorak. (Dvorak 1986) Other chronic inflammatory conditions that are recognized as being associated with an increased risk of cancer include schistosomiasis (bladder) and ulcerative colitis (colon).

2. INFECTIOUS CAUSES

The relatively recent realization that many chronic bacterial and viral infections are also associated with tumour formation provides the most compelling examples. In addition to schistosomiasis and bladder cancer, the more recently discovered *Helicobacter pylori*, originally associated with chronic gastritis, is now recognized as a causative agent for gastric adenocarcinoma and gastric mucosal-associated lymphoid tissue lymphomas (Williams and Pounder, 1999).

2.1 Virus association

Perhaps the strongest association between chronic infection and the development of cancer is that between chronic viral infections and tumour induction. (Dalgleish, 1991). The best examples are those of chronic hepatitis B and hepatitis C virus infection, both of which cause chronic hepatitis from which primary liver cancers evolve, often decades later. Similarly, chronic human papilloma virus infection is clearly associated with inflammatory changes in the cervix leading to cervical cancer and cancers of the perineal region. Epstein Barr Virus (EBV), which exists in a dormant
state in the vast majority of the Western population, is able to cause Burkitt’s lymphoma in Africa, where the additional stimulus is chronic immune activation, probably by malaria. Similarly, it is associated with nasopharyngeal carcinoma, particularly in the Far East where chronic irritation/inflammation/immune activation by salted fish stimulates EBV replication leading to these unusual tumours. EBV is largely asymptomatic but is clearly able to cause lymphomas, particularly in immunosuppressed patients, such as post transplant patients or acquired immune deficiency syndrome (AIDS) patients.

The most recent example of a virus causing a tumour is the discovery of the new herpes sarcoma virus or human herpesvirus 8 (HHV-8), which is the causative agent of Kaposi’s sarcoma (Brooks et al., 1997). There is a strong association with HIV and this cancer may undergo spontaneous resolution with treatment leading to a reduction of HIV load. HIV is associated with chronic immune activation, which is reduced when the viral load is lowered.

2.2 The requirement for chronic inflammation

Although the viruses above have well described oncogenic properties, they rarely cause disease in the absence of chronic immune activation or inflammation. The fact that chronic irritation/inflammation does not need to be caused by an infectious agent in order to increase the propensity for cancer to develop is clearly shown by tobacco-related cancers. Lung cancer, for instance, is clearly associated with smoking and it is dependent on the amount smoked (packs per day) as well as the number of years smoked. Many of the patients suffer from chronic bronchitis for a number of years and even in those patients who do not have overt bronchitis, histology of non macroscopically tumour-free bronchi show chronic inflammatory changes. Other chronic irritants also lead to inflammation and subsequent tumours, such as asbestos in mesothelioma. Inappropriate enzymatic exposure can also result in chronic inflammation. For example, reflux esophagitis and Barratt’s esophagitis are both associated with the development of esophageal adenocarcinoma. For a full list of associations of cancer or recognized associations of cancer and chronic infection/irritation/inflammation see Table 1.

The association between chronic inflammation and tumour formation strongly suggests that sites of chronic inflammation are ideal microenvironments for cancer to evolve (Table 1). Here, the similarity with wound healing is pertinent as in wounds, cell-mediated immunity is suppressed, presumably to prevent breaking of tolerance of “self” tissue as it is being repaired. The second component is the induction of angiogenesis, being new blood vessel formation which in itself is associated with increased
Table 1. Relationship between known causes of Chronic Inflammation and Cancer

<table>
<thead>
<tr>
<th>Causative inflammatory stimulus</th>
<th>Cancer</th>
<th>Mechanism of action and precursor states</th>
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<tbody>
<tr>
<td>EBV</td>
<td>Burkitts Lymphoma (BL)</td>
<td>Associated with chronic immune activation such as malaria in BL and smoked food in NPC</td>
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<tr>
<td></td>
<td>Nasopharyngeal (NPL)</td>
<td></td>
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<td></td>
<td>Post transplant lymphoma</td>
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<td></td>
<td>Immunosuppression associated lymphoma</td>
<td></td>
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<tr>
<td></td>
<td>NHL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Breast cancer</td>
<td></td>
</tr>
<tr>
<td>HBV/HCV</td>
<td>Viruses induce hepatitis followed by cirrhosis (angiogenesis) followed by oncogenic transformation - hepatic cancer</td>
<td>Aflatoxin may enhance the degree of inflammation</td>
</tr>
<tr>
<td>HPV</td>
<td>Cervix/anal/perineum/?upper aerodigestive track</td>
<td>May require extra exogenous causative agent of cervicitis to progress, e.g. chlymidia, and/or immunosuppression</td>
</tr>
<tr>
<td>HHV-8</td>
<td>Kaposi’s Sarcoma</td>
<td>Only in presence of immunosuppression due to age (mild) or HIV infection (aggressive) which causes marked immune activation</td>
</tr>
<tr>
<td>HIV</td>
<td>Lymphoma (EBV driven)</td>
<td>HIV induces immunosuppression as well as chronic immune activation which appears dependant on the immunogenetics of host</td>
</tr>
<tr>
<td></td>
<td>Kaposi’s Sarcoma (HHV-8 driven)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cervix (HPV driven)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(HIV is not oncogenic directly in contrast to the above)</td>
<td></td>
</tr>
<tr>
<td>HTVL-1</td>
<td>T-cell lymphomas and leukaemia</td>
<td>Causes chronic T-cell activation</td>
</tr>
<tr>
<td><em>Helicobacter pylori</em></td>
<td>Stomach cancer</td>
<td>Gastritis/ulcers</td>
</tr>
<tr>
<td></td>
<td>Lymphoma of gut (MALT)</td>
<td></td>
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<tr>
<td>Schistosomiasis</td>
<td>Bladder</td>
<td>Chronic cystitis</td>
</tr>
<tr>
<td>Tobacco smoke Nicotine Infections</td>
<td>Lung cancer</td>
<td>Chronic bronchitis Inflammation of tunica medica</td>
</tr>
<tr>
<td>Asbestos</td>
<td>Mesothelioma</td>
<td>Asbestos, fibrotic, plaques</td>
</tr>
<tr>
<td>Ulcerative colitis Crohn’s ? bile salts</td>
<td>Colorectal cancer</td>
<td>Causes of inflammatory bowel disease including polyps and adenomas</td>
</tr>
<tr>
<td>Reflux +?</td>
<td>Oesophageal cancer</td>
<td>Oesophagitis/obesity/tobacco/nicotine</td>
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<tr>
<td>Prostatitis +?cause</td>
<td>Prostate cancer</td>
<td>Infectious cause</td>
</tr>
<tr>
<td>Chronic pancreatitis +?cause</td>
<td>Pancreatic cancer</td>
<td>Causative agent unclear</td>
</tr>
<tr>
<td>UV light</td>
<td>Melanoma</td>
<td>Skin inflammation and immunosuppression</td>
</tr>
<tr>
<td>Chronic tar/soot irritation</td>
<td>Scrotum</td>
<td>Common in chimney sweeps in Victorian era</td>
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</tbody>
</table>
amounts of growth factors. If continued indefinitely, as in chronic inflammation, this environment would be ideal for cancer to develop. It is broadly recognized that cancer is a sequence of stochastic events involving permanent activation of oncogene pathways and deletion of tumour suppressor genes and their pathways. On average, six such events are required to occur for cancer to develop. It has been shown that single mutations in oncogenes can be recognized by cytotoxic T lymphocytes suggesting that in the absence of suppression of the immune system, a cell developing such a mutation would induce an immune response against this new epitope and the cell would be killed. In a background of chronic inflammation, immune induction does not occur and the cell is able to survive long enough to develop the next random event. When the tumour does start to develop, it has an environment full of growth factors and the ability to establish new vasculature.

The most important impact of the association between chronic inflammation and cancer is that treatment of chronic inflammation should lead to a lower incidence of cancer. This is one of the most convincing aspects of the association as there are numerous studies showing that the daily use of anti-inflammatories, such as aspirin, other NSAIDS, and cyclooxygenase (COX)-2 inhibitors, are able to reduce the incidence of colorectal cancer by up to half. In addition, the incidence of other solid tumours are also reduced by a significant amount (Harris et al., 2005).

3. CANCER AND THE IMMUNE RESPONSE

T cells produce (at least) two major cytokine patterns. The first is generated by Th-1 cells, which produce interleukin (IL)-2, interferon (IFN)-γ, and IL-12 and affect the cell mediated immune (CMI) response, and the second by Th-2 cells, which release IL-4, IL-5, IL-6, and IL-10 and affect the hormonal response (HIR). This discovery, initially in mice but later also in humans, has had a major impact on understanding the complex changes and interactions between an infectious agent and the immune system (Mosman and Coffman, 1989). Th-1 cytokines are required for a strong CMI response and are decreased in many chronic infectious diseases, including AIDS, tuberculosis (TB), and many tropical infections. In what might appear a compensatory response, Th-2 cytokines are associated with humoral immunity (HI) and often increase as is the case in diseases where CMI is suppressed, e.g., AIDS and tuberculosis.

Many malignancies are associated with some degree of suppression of CMI responses (Lee et al. 1997; Maraveyas et al., 2000; Pettit et al., 2000). Cancers use a wide variety of methods to evade an immune response including downregulation of molecules of the major histocompatibility
complex (MHC, HLA in humans) and upregulation of ligands that kill engaging killer T-cells, i.e. CD95L and DC178. The production of immunosuppressive cytokines by tumours, such as TGFβ and IL-10 could account for much of the inhibition of CMI (Doherty et al., 1994; Ganss and Hanahan, 1998; Garrido et al., 1993; Gorter and Meri, 1999; Melief and Kast, 1991; Strand and Galle, 1998; Pettit et al., 2000).

The suppression of local CMI responses has been reported in a number of studies evaluating inflammatory cellular infiltrates in tumours from patients with malignant cancers including non-small cell lung cancer (NSCLC), head and neck, oesophagus, breast and genitourinary cancers, lymphomas and sarcomas (Asselin-Paturel et al., 1998; O’Sullivan et al., 1996; Hildesheim et al., 1997; Lee et al., 1997; Aziz et al., 1998; O’Hara et al., 1998) as well as carcinoma in situ including Barrett’s oesophagus and cervical intraepithelial neoplasia (CIN) (Hildesheim, et al. 1997; Oka, et al. 1992; Sonnex, 1998). Systemic immunosuppression can be documented by looking at intracellular cytokine production following stimulation of lymphocytes in vitro and has been shown in several cancer types including melanoma and colorectal cancer (Maraveyas et al., 1999; Heriot et al., 2000). The presence of a dominant Th-2 immune response in potentially curable tumours, such as lymphomas, is associated with a fatal outcome (Lee et al., 1997). Absent or reduced delayed hypersensitivity reactions to common T-cell recall antigens are a manifestation of CMI. These responses are either reduced or absent in many pre-malignant and malignant tumours including CIN, Hodgkin’s disease, gastric carcinoma, small cell lung cancer, and malignant melanoma (Roses et al., 1979; Lang et al., 1980; Johnston-Early et al., 1983; Richtsmeier and Eisele, 1986; Cainzos, 1989; Hopfl et al. 2000).

T-cell anergy is commonly seen in malignant disease. A number of processes may be responsible for this. T-cells from cancer patients have abnormalities in their signal transduction pathways. The T-cell receptor (TCR)-αβ or -γδ chains bind the peptide ligand while, in turn, the TCR is coupled to intracellular signal transduction components by TCR-ζ subunits. The TCR-associated signalling molecule CD3 is made up of a number of components which stabilize surface expression of the TCR and are essential for interaction with MHC-antigen complexes. The T-cell alterations found in in vivo models of malignant disease include complete absence of CD3-ζ which is replaced by the Fc εγ-chain and a reduction in the ability of T lymphocytes to produce the Th-1 cytokines IL-2 and IFN-γ (Mizoguchi et al., 1992; Salvadori et al., 1994; Zea et al., 1995).

In malignant mesothelioma, the relative CDδ, CDγ and CDζ mRNA levels expressed by tumour infiltrating lymphocytes (TILs) decrease. In addition, transforming growth factor-β (TGFβ), a potent tumour cell growth and immunosuppressive factor, is produced. (A feature which is not limited
Inflammation and Cancer

In this disease, however, the suppression of CD3 subunit expression with resultant functional impairment of TILs is reversed in vivo by inducing TGFβ antisense RNA. This indicates that TILs are deactivated by tumour-associated immunosuppressive factors upon infiltration of the tumour microenvironment (Jarnicki et al., 1996).

COX enzymes are responsible for the synthesis of prostaglandins, the precise prostaglandin synthesized depending on the prostaglandin synthase enzyme present in the cell. COX-2, the inducible form of the enzyme, is constitutively expressed in virtually all premalignant and malignant cancers, including colorectal, upper gastrointestinal tract, pancreatic, head and neck, lung and breast cancers (Murata et al., 1999; Koshiba et al., 1999; Mestre et al., 1999; Molina et al., 1999; Wolff et al., 1998; Huang et al., 1998; Taketo, 1998; Tsujii et al., 1997; Uotila, 1996; Vainio and Morgan, 1998; Vane et al., 1998). More recently, COX-2 expression has been shown to correlate with local chronic inflammation and tumour neovascularisation in human prostate cancer (Wang, et al. 2005). It is particularly associated with prostaglandin E2 (PGE2). On binding to its receptor on T-cells, PGE2 induces cyclic adenosine monophosphate (cAMP) which inhibits the proliferation of Th-1/CMI-associated CD4+ lymphocytes while stimulating the proliferation of Th-2 CD4+ lymphocytes resulting in avoidance of immunesurveillance. The importance of the Th-1/CMI response in both tumour regression and rejection underscores the significance of these changes. Tumour-specific cytotoxic T-cells represent a major effector arm of the Th-1/CMI response as demonstrated by studies of adoptive T-cell transfer (Greenberg, 1991; Papadopoulos et al., 1994; Rooney et al., 1995). However, the presence of such effector cells is only seen in a small minority of cases in the setting of tumour progression. Both Th-1 and Th-2 cytokine gene transduction experiments in animal tumour cell lines have resulted in CMI responses capable of inhibiting a subsequent challenge with parental tumour cells. Moreover, in order to induce established tumour regression, Th-1 cytokine secreting effector cells are required (Forni and Foa, 1996), and where tumour rejection occurs, the induction of tumour-specific CMI responses is generally seen. Collectively these findings indicate that tumour growth either fails to stimulate an effective CMI response or evades immunesurveillance at least in part through inhibition of TIL CMI functions both locally and systemically (Browning and Bodmer, 1992; Jarnicki et al., 1996).

Malignant melanoma is a highly metastatic cancer of the melatonin-producing cells of the skin and is notoriously resistant to classical treatments such as chemotherapy and radiotherapy. Employing the same fluorescence-activated cell sorting (FACS) techniques used to detect intracellular cytokines in AIDS patients, a significant reduction in Th-1 cytokine
production can be found in these patients (Maraveyas et al., 1999). For many years, however, it has been recognised that this cancer is sensitive to a variety of immunology-based therapies which act by boosting CMI responses. Skin lesions often disappear following direct intralesional administration of Bacillus Calmette-Guerin (BCG) vaccine. Similar responses have been seen with cell-based vaccines or lysates, melanoma specific peptides, either given alone or pulsed onto dendritic cells. Successful treatment with immunotherapy, resulting in either stable disease or an objective tumour response has been found to be associated with a switch from a Th-2/HI dominant profile to a Th-1/CMI dominant one (Grange et al., 1995; Sredni et al., 1996; Hu et al., 1998; Hrouda et al., 1998; Dalgleish, 1999).

The impact of a reduced CMI response being induced by a tumour is illustrated by colorectal cancer in which even in patients with early small volume (Duke’s A and B) tumours, a reduction in systemic Th-1-like responses is seen compared to age- and sex-matched controls without disease (Heriot et al., 2000). In the latter case, the observation that these responses return to normal following surgery strongly supports the deduction that it is the tumour that causes the reduction in CMI responses. More recently, the same patients have been reanalysed and the level of Th-1 responses have been found to correlate with survival irrespective of subsequent treatment (Charles Evans and co-workers, unpublished data).

In NSCLC malignant pleural effusions, the majority of lymphocytes are T-cells with a Th-2 phenotype whilst less than 1% are natural killer cells. Following Th-1 cytokine therapy with IL-2 and IL-12, the T-helper lymphocytes shift to a Th-1 phenotype. The specific anti-tumour cytotoxic property of these T-lymphocytes can be restored by the use of IL-2 treatment and TCR-CD3 engagement. IL-2 and IL-12 act synergistically in this setting (Chen et al., 1998; Chen et al., 1997b).

Gene knockout experiments have provided the most conclusive evidence for an association between deficient Th-1 responses and a predisposition to cancer. An increased incidence of solid tumours is seen in IFN-γ, IFN-γ receptor or signal transducer and activator of transcription (STAT) 1 (a component of the IFN signalling pathway) knockout mice (Chen et al., 1998; Kaplan et al., 1998). Therefore CMI suppression may provide the ideal environment for cancer cells to develop and grow. A single mutation in an oncogene would probably be identified by a cytotoxic T lymphocyte in a normal environment but cells carrying such a mutation are able to survive in a privileged, depressed CMI immune response site. As a result, the mutation may persist leaving the cells’ DNA primed for another stochastic event to occur, such as a p53 mutation.
As the neoplastic lesion grows, it becomes progressively hypoxic. Hypoxia is associated with suppression of CMI responses (Lee et al., 1998; Sairam et al., 1998) which in turn would allow escape of the malignant process from immunesurveillance. Collectively these findings indicate that effective reversion of immune tolerance may have a role to play not only in the treatment of established malignant disease but also in chemoprevention.

Exposure to a foreign antigen results in upregulation of the non-specific pro-inflammatory cytokines IL-1α and β and the Th-1 cytokines in inflammatory cells. COX-1 and -2 are among the most important enzymes in the regulation of the immune response and play a key role in angiogenesis, the inhibition of apoptosis, cell proliferation and motility. COX-1 is constitutively expressed by many cells. In contrast COX-2 is produced by epithelial, mesenchymal and inflammatory cells following exposure to pro-inflammatory cytokines (Taketo, 1998; Uotila, 1996; Vane et al., 1998), which are induced by infective agents and environmental factors known to be associated with the development of malignant disease including *Helicobacter pylori* infection (Sawaoka et al., 1998a), nicotine (Schror et al., 1998), and tobacco-specific nitrosamine 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanone (NNK) (El-Bayoumy et al., 1999). Overexpression of COX-2 is sufficient to induce tumorigenesis in the mammary glands of transgenic mice derived using the murine mammary tumour virus promoter (Liu et al., 2001). Th2 cytokines, such as IL-4 and IL-10, which can inhibit the synthesis of Th-1 cytokines by CD4⁺ T-helper lymphocytes, are produced in COX-2 expressing environments. These Th-2 cytokines not only downregulate both pro-inflammatory/Th-1 cytokines but also COX-2 expression itself (Della Bella et al., 1997; Subbaramaiah et al., 1997; Uotila, 1996; Vane et al., 1998) (Fig. 1). Chronic antigen exposure may drive a continuous cycle in which induced pro-inflammatory and Th-1 cytokines upregulate COX-2 leading to chronic HI/Th-2 cytokine production and subsequent impairment of the CMI response. In predisposed individuals, this cycle may eventually lead to a predominant HI response environment. The importance of pro-inflammatory cytokines driving the HI response is underpinned by the observation that TNF-deficient mice are resistant to skin carcinogenesis (Moore et al., 1999).

The results of these studies consistently demonstrate that not only is cancer itself associated with a shift from a Th-1 to a Th-2 dominant phenotype but that conditions predisposing to malignant disease likewise 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.
Figure 1. Exposure to carcinogenic stimuli results in upregulation of cell survival factors in affected cells including cyclooxygenase (COX)-2. COX-2 plays a key role in the conversion of arachidonic acid to prostaglandins including PGE2. PGE2 downregulates the synthesis of Th1 cytokines and upregulates Th-2 cytokines in inflammatory and/or affected epithelial, mesenchymal or haematopoietic cells resulting in suppressed cell mediated immune responses (CMI), increased angiogenesis and inhibition of apoptosis. In an acute exposure situation the feedback between the initial pro-inflammatory response and the anti-inflammatory Th2 cytokines is self-limiting. However in the case of cancer associated chronic immune activation conditions sustained exposure to the antigen/chemical drives the cycle continuously resulting in a sustained pre-dominant Th2 immune response, angiogenesis and inhibition of apoptosis facilitating the development of cancer in a pre-disposed individual.

4. INITIAL INFLAMMATION RESPONSE: KILL OR CURE

The difference between a pro-inflammatory response to an antigen or irritant and the type of immune response established is crucial as to whether the cancer is fed by inflammation or is rejected by it. There are numerous factors which can determine whether an immune response favours cancer progression or its elimination. There are several studies showing that tumour infiltrating lymphocytes are a good prognostic factor. Indeed, reculturing these lymphocytes and expanding them \textit{ex vivo} before re-infusion with IL-2
was reported as being capable of inducing clinical responses (Rosenberg et al., 1986). However, it is now apparent that non-infiltrating immune cells are exhibiting anti-tumour activity. Tumour-associated macrophages (TAMs) have been shown to correlate with vessel density in a number of malignancies and the associated expression of epidermal growth factor (EGF) and EGF receptor (EGFR) in cancer cells. This is associated with reduced patient survival. Co-culture of cancer cells with macrophages can actually enhance cancer cell invasive potential and matrix degrading activity and upregulate pro-tumorigenic genes. Chen et al. have shown that this activity can be reduced with anti-inflammatory drugs, including Thalidomide (Chen et al., 2005). Whether the macrophages have a positive or negative effect on tumour growth clearly depends on the tumour microenvironment and the stroma involved. Indeed, different results reported in the literature would appear to be dependent on whether the macrophages are predominantly in the tumour islets or in the stroma. Thus, it would appear that macrophages predominantly in the stroma are pro-tumorigenic, whereas macrophages predominant in the tumour islet are associated with a significant survival time. Indeed, Tomas Walsh and colleagues (personal communication) have noted that patients with a high islet macrophage density but incomplete resection have an overall longer survival than patients with low islet macrophage density but complete resection.

What determines the result of this interaction is unclear but would appear to involve chemokines and their receptors as most cancers express an extensive network of these. Tumour-associated chemokines are thought to control leukocyte infiltration into the tumour, the immune response against it, the regulation of angiogenesis and the control of metastatic spread. Chemokines can influence the distribution of the immune response, including lymphocytes, monocytes/macrophages and pre-dendritic cells. They are also able to promote angiogenesis and may also contribute directly to the transformation of cells by acting as growth and survival factors. Moreover, they are crucial to the spread of cells. Indeed, in mouse models, the level of expressed tumour-derived chemokines determines whether macrophage infiltration is pro-tumorigenic or capable of destroying tumour cells. Manipulating chemokine levels and their receptors clearly could have a major role in the treatment of cancer. In order to reject a tumour, an acute, as opposed to a chronic inflammatory state needs to be introduced. Here, it is clear that the interplay between innate and adaptive immunity and, in particular, the interactions between NK and dendritic cells are of vital importance for effective tumour control (de Visser and Coussens, 2005). The importance of the immune response in controlling the development of cancer can only be fully appreciated when considering the numerous methods
employed by tumours to escape immune control (reviewed by Igney and Krammer, 2005).

5. THE INTER-RELATIONSHIP BETWEEN THE IMMUNE RESPONSE AND ANGIOGENESIS

The relationship between the immune response and angiogenesis (O’Byrne et al., 2000a) is important in its own right with regard to fostering tumour growth. Angiogenesis, the formation of a new blood supply from an existing vasculature, is necessary for the development of early neoplastic lesions and the growth of invasive and metastatic disease. This process occurs in all tumours and is under the regulation of pro-angiogenic factors including Th-2 cytokines such as IL-6 and vascular endothelial growth factor (VEGF). The intensity of the angiogenic process, as assessed by microvessel counting methods, correlates with primary tumour growth, invasiveness, and metastatic spread of the disease (Folkman, 1995; O’Byrne et al., 2000b). Furthermore, there is a strong correlation between tumour cell expression of angiogenic growth factors, such as VEGF and angiogenesis, and patient outcome (O’Byrne et al., 2000b).

Recent research indicates that normal physiological processes which require angiogenesis, such as ovulation, implantation into the ovary and wound healing, occur in a HI-predominant environment (Folkman, 1995; Richards et al., 1995; Kodelja et al., 1997; Piccini et al., 1998; Schaffer and Barbul, 1998; Singer and Clark, 1999). HI-stimulated macrophages induce endothelial cell proliferation 3 to 3.5 times more than CMI stimulated macrophages in coculture experiments (Kodelja et al., 1997). These findings are supported by work in IL-6 knockout mice where the capacity to heal wounds and regenerate normal hepatic tissue, both processes which require angiogenesis, is impaired (Gallucci et al. 2000; Wallenius et al., 2000). In contrast to the upregulated HI immune response seen, CMI responses are suppressed during ovulation, implantation, and wound healing. This prevents rejection of sperm and embryo, and presentation of damaged tissues to the immune system as non-self, which might induce an autoimmune response to healing or healed tissues (Richards et al., 1995; Piccini et al., 1998; Schaffer and Barbul, 1998; Singer and Clark, 1999). In contrast to HI immune response-induced angiogenesis, CMI immune responses tend to inhibit angiogenesis (Watanabe et al., 1997). B lymphocytes, which are synonomous with HI have been shown to be vital in promoting chronic inflammation-dependent de novo carcinogenesis (de Visser et al., 2005).

Unlike normal physiological processes, the factors that suppress CMI and switch on angiogenesis persist in many established chronic infectious/inflammatory states, particularly conditions associated with the
subsequent development of malignant disease (Fig. 2). These include chronic viral infections (see below), asbestos (Bielefeld-Omann et al., 1996) and cigarette smoke (Mayne et al., 1999). Chronic exposure to cigarette smoke leads to chronic obstructive pulmonary disease (COPD) in predisposed individuals. COPD is an independent predictor for the development of lung cancer (Mayne et al., 1999). In keeping with this, inflamed lung mucosa has increased vascularity compared with uninflamed mucosa (Fisseler-Eckhoff et al., 1996). Furthermore, bronchial dysplasia and carcinoma in situ, precursors to the development of malignant disease, have increased vascularity compared to normal bronchial epithelium (Fontanini et al., 1996; Fisseler-Eckhoff et al., 1996). Using fluorescent bronchoscopy, angiogenic squamous dysplastic lesions have been identified in 34 percent of high risk smokers without carcinomas and in 60 percent of patients with squamous cell lung carcinoma (Keith et al., 2000). Cigarettes contain a number of factors, including nicotine, which may predispose to the development of malignant disease. Nicotine induces angiogenesis and reduces CMI which would facilitate the survival and proliferation of a cell transformed by carcinogens such as NNK (Heeschen et al., 2001).

Figure 2. The inflammatory environment resulting in angiogenesis and growth factors, as well as reduced cell mediated immune surveillance, provides the ideal environment for a mutant cell to evolve through the six or more minimum changes required for metastatic cancer to develop. These features help both initiation and promotion resulting in a tumour that tries to replicate this favourable environment by secreting angiogenic and immunosuppressive factors.
If this state occurs for several years, then random mutations in the cells of the affected tissues, caused by carcinogens or unregulated proliferation, would occur not only in an immunologically tolerant, but also a microvesSEL-rich environment. Phenotypic changes, e.g. proteins resulting from mutations in the ras oncogene, which would normally be detected by cytotoxic lymphocytes, may escape immune surveillance. At the same time, these cells would have an adequate supply of oxygen and nutrients as well as clearance of waste metabolic products allowing another step in the stochastic progression towards malignancy to occur (Gjertsen et al., 1997). Indeed, it is so important to maintain this environment that developing neoplastic cell clones evolve to mimic this state in order to progress and metastasise. This contention is supported by the fact that tumours secrete CMI immunosuppressive cytokines such as TGFβ and IL-10 (Kiessling et al., 2000).

Again, induction of COX-2 may be central to the development of an angiogenic environment in many of the conditions leading to the subsequent development of malignancy. COX-2 expressing tumour cells are associated with the production of a number of angiogenic growth factors and the synthesis and activation of matrix metalloproteinases (MMPs), both of which favour tumour invasion and angiogenesis (Tsujii et al., 1997; Tsujii et al., 1998; Takahashi et al., 1999). Cigarette smoke carcinogens, including the tobacco-specific carcinogen NNK (which reproducibly induces pulmonary adenocarcinomas in laboratory rodents) and nicotine are associated with COX-2 upregulation. NNK, which is a beta-adrenergic receptor agonist, does so by releasing arachidonate and nicotine acts through nicotinic acetylcholine receptors (Saareks et al., 1998). Inhibition of COX-2 activity reduces IL-6 and IL-8 levels secreted by human cell lines further supporting the strong link between inflammation, HI cytokine expression and angiogenesis (Luca et al., 1997; Salgado et al., 1999; Hong et al., 2000).

6. INFLAMMATION AND APOPTOSIS

Chronic inflammation also gives rise to the production of growth factors and cytokines, and the activation of intracellular cell survival pathways that would result in inhibition of apoptosis. An example of such a factor released during inflammatory states is macrophage inhibitory factor (MIF), which has recently been shown to repress the transcription activity of p53 and its downstream targets p21 and bax, thereby having a marked anti-apoptotic effect (Cordon-Cardo and Prives, 1999; Hudson et al., 1999). Experimental evidence indicates that p53 plays an important role in the mediation of Th-1 cytokine-induced cytotoxicity (Yeung and Lau, 1998; Das et al., 1999; Kano et al., 1999; Um et al., 2000; Takagi et al., 2000). p53 induces Transporter Associated with Antigen Processing (TAP) 1 expression through a p53-
responsive element. TAP1 is required for the MHC class I antigen presentation pathway. p73, which is homologous to p53, also induces TAP1 and cooperates with p53 to activate TAP1. Through the induction of TAP1, p53 enhances the transport of MHC class I peptides and expression of surface MHC-peptide complexes. p53 cooperates with IFN-γ to activate the MHC class I pathway. These results indicate that, as part of their function as tumour suppressors, p53 and its homologue p73 may play a role in tumour surveillance (Zhu et al., 1999). Therefore, inflammation is capable of inactivating one of the most important cell regulatory pathways controlling cancer development, reducing the effectiveness of the body's own cellular defense reaction to a mutation. p53 also has a key role in the regulation of angiogenesis, in part through induction of the anti-angiogenic factor thrombospondin (Dameron, 1994). Furthermore, mutations of p53 may result in induction of the synthesis of the most potent angiogenic agent known, VEGF, leading to increased angiogenesis (Kieser et al., 1994; Volpert et al., 1997). Therefore, loss of p53 would also result in an impaired CMI response, facilitate angiogenesis, and result in a loss of apoptotic activity. Mutations of the ras oncogene can induce IL-8 and induce stromal inflammation that can lead to cancer progression (Sparmann and Bar-Sagi, 2004).

There is thus increasing evidence that exposure to carcinogens, such as ultraviolet light (UV) (Athar et al., 1989), the tobacco-specific carcinogen NNK (El-Bayoumy et al., 1999), nicotine (Saareks et al., 1998), Helicobacter pylori (Konturek et al., 2000; Sawaoka et al., 1998a) and colonic luminal contents, in particular the dihydroxy bile acids deoxycholate and chemodeoxycholate (Zhang et al., 2000; Glinhammar et al., 2001) all upregulate COX-2 in the affected tissue. In both non-neoplastic and neoplastic cells, COX-2 is associated with cell proliferation (Tsuji et al., 1996; McGinty et al., 2000) and inhibition of apoptotic activity, at least in part through the induction of bcl-2 (Tsuji and DuBois, 1995).

The serine/threonine kinase Akt (protein kinase B) is activated in response to a variety of stimuli. This factor provides a survival signal that protects cells from apoptosis induced by growth factor withdrawal. Through the phosphorylation of specific targets such as Bad (del Peso et al., 1997) and procaspase-9 (Cardone et al., 1998), the Akt cell survival signalling pathway inhibits apoptosis. Some carcinogens act, at least in part, by inducing oxidative stress in exposed cells. Oxidative stress results in the activation of intracellular survival cell signalling pathways. In a variety of cell types, H2O2, an inducer of oxidative stress, has been shown to induce elevated Akt activity in a time- and dose-dependent manner by a mechanism involving phosphoinositide 3-kinase (PI3K). Inhibitors of PI3K activity, including wortmannin and LY294002, and expression of a dominant negative mutant of p85, a regulatory component of PI3K, inhibited H2O2...