Paradoxical roles of the immune system during cancer development

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Abstract | The main function of the mammalian immune system is to monitor tissue homeostasis, to protect against invading or infectious pathogens and to eliminate damaged cells. Therefore, it is surprising that cancer occurs with such a high frequency in humans. Recent insights that have been gained from clinical studies and experimental mouse models of carcinogenesis expand our understanding of the complex relationship between immune cells and developing tumours. Here, we examine the paradoxical role of adaptive and innate leukocytes as crucial regulators of cancer development and highlight recent insights that have been gained by manipulating immune responses in mouse models of *de novo* and spontaneous tumorigenesis.

Self-antigens
Antigens that are derived from normal, unaltered proteins that are expressed in tissues. The immune system does not respond to self-antigens because of immune-tolerance mechanisms; however, under certain circumstances, adaptive immune responses can be elicited towards self-antigens and result in autoimmune disease.

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Cancer is an insidious disease that originates from mutant DNA sequences that reroute crucial pathways regulating tissue homeostasis, cell survival and/or cell death. In recent decades, much has been learned by studying homogeneous populations of tumour cells that harbour activating or inactivating genetic mutations; however, cancers are not merely autonomous masses of mutant cells. Instead, cancers are composed of multiple cell types, such as fibroblasts and epithelial cells, innate and adaptive immune cells, and cells that form blood and lymphatic vasculature, as well as specialized mesenchymal cell types that are unique to each tissue microenvironment. Whereas tissue homeostasis is maintained by collaborative interactions between these diverse cell types, cancer development is enhanced when mutant cells harness these collaborative capabilities to favour their own survival.

How do survival-advantaged mutant cells neutralize homeostatic growth constraints and develop into cancerous masses that not only induce primary organ dysfunction, but also relocate within the organism and often cause lethal complications? Recent mechanistic studies, in combination with a vast amount of clinical literature, support the contention that cancer development largely depends on the ability of mutant cells to hijack and exploit the normal physiological processes of the host. As we are now recognizing, each stage of cancer development is exquisitely susceptible to regulation by immune cells (BOX 1). Whereas full activation of adaptive immune cells in response to the tumour might result in eradication of malignant cells, chronic activation of various types of innate immune cells in or around pre-malignant tissues might actually promote tumour development. Here, we review the paradoxical relationship of innate and adaptive immune cells with cancer and highlight recent insights that have been gained by manipulating immune responses in mouse models of *de novo* and spontaneous tumorigenesis.

Immune cells and tissue homeostasis

The mammalian immune system is composed of many cell types and mediators that interact with non-immune cells and each other in complex and dynamic networks to ensure protection against foreign pathogens, while simultaneously maintaining tolerance towards self-antigens. Based on antigen specificity and timing of activation, the immune system is composed of two distinct compartments — adaptive and innate. Whereas the cellular composition and antigen specificity of these are distinct, they have each evolved sophisticated communication networks that enable rapid responses to tissue injury. Innate immune cells, such as dendritic cells (DCs), natural killer (NK) cells, macrophages, neutrophils, basophils, eosinophils and mast cells, are the first line of defence against foreign pathogens. DCs, macrophages and mast cells serve as sentinel cells that are pre-stationed in tissues and continuously monitor their microenvironment for signs of distress.

When tissue homeostasis is perturbed, sentinel macrophages and mast cells immediately release soluble mediators, such as cytokines, chemokines, matrix remodelling proteases and reactive oxygen species (ROS), and bioactive mediators such as histamine, that induce mobilization and infiltration of additional

At a glance

- Adaptive and innate immune cells regulate tissue homeostasis and efficient wound healing.
- Altered interactions between adaptive and innate immune cells can lead to chronic inflammatory disorders.
- In cancers, an abundance of infiltrating innate immune cells, such as macrophages, mast cells and neutrophils, correlates with increased angiogenesis and/or poor prognosis.
- In cancers, an abundance of infiltrating lymphocytes correlates with favourable prognosis.
- Chronic inflammatory conditions enhance a predisposition to cancer development.
- Long-term usage of non-steroidal anti-inflammatory drugs and selective cyclooxygenase-2 inhibitors reduces cancer incidence.
- Polymorphisms in genes that regulate immune balance influence cancer risk.
- Immune status in humans and in mouse models affects the risk of cancer development in an aetiology-dependent manner.
- Genetic elimination or depletion of immune cells alters cancer progression in experimental models.
- Activation of antitumour adaptive immune responses can suppress tumour growth.

leukocytes into damaged tissue (a process that is known as inflammation). Macrophages and mast cells can also activate vascular and fibroblast responses in order to orchestrate the elimination of invading organisms and initiate local tissue repair. DCs, on the other hand, take up foreign antigens and migrate to lymphoid organs where they present their antigens to adaptive immune cells. They are, therefore, key players in the interface between innate and adaptive immunity.

NK cells also participate in cellular crosstalk between innate and adaptive immune cells through their ability to interact bidirectionally with DCs; certain NK-cell subsets eliminate immature DCs, whereas others promote DC maturation, which can then also reciprocally regulate activation of NK cells[1–3]. The unique characteristic of innate immune cells — their inherent ability to rapidly respond when tissue injury occurs, without memory of previous assaults or antigen specificity — is a defining feature that sets them apart from adaptive immune cells.

Acute activation of innate immunity sets the stage for activation of the more sophisticated adaptive immune system. Induction of efficient primary adaptive immune responses requires direct interactions with mature antigen-presenting cells and a pro-inflammatory milieu. Adaptive immune cells, such as B lymphocytes, $CD4^+$ helper T lymphocytes and $CD8^+$ cytotoxic T lymphocytes (CTLs), distinguish themselves from innate leukocytes by expression of somatically generated, diverse antigen-specific receptors, which are formed as a consequence of random gene rearrangements and allow a flexible and broader repertoire of responses than innate immune cells, which express germline-encoded receptors.

As individual B and T lymphocytes are antigenically committed to a specific unique antigen, clonal expansion upon recognition of foreign antigens is required to obtain sufficient antigen-specific B and/or T lymphocytes to counteract infection. Therefore, the kinetics of primary adaptive responses are slower than innate responses. However, during primary responses a subset of lymphocytes differentiate into long-lived memory cells, resulting in larger responses upon subsequent exposure to the same antigen.

Together, acute activation of these distinct immune-response pathways efficiently removes or eliminates invading pathogens, damaged cells and extracellular matrix (ECM). In addition, once assaulting agents are eliminated, immune cells are crucially involved in normalizing cell-proliferation and cell-death pathways to enable re-epithelialization and new ECM synthesis. Once wound healing is complete, inflammation resolves and tissue homeostasis returns. Because of their enormous plasticity, immune cells exert multiple effector functions that are continually fine-tuned as tissue microenvironments are altered. Therefore, the immune system is integrally involved in maintaining tissue homeostasis as well as being implicated in the pathogenesis of many chronic diseases, such as arthritis, heart disease, Alzheimer disease and cancer[4].

Box 1 | **Mechanisms by which immune cells regulate cancer development**

Mechanisms by which innate immune cells* contribute to cancer

Direct mechanisms

- Induction of DNA damage by the generation of free radicals.
- Paracrine regulation of intracellular pathways (through nuclear factor κB).

Indirect mechanisms

- Promotion of angiogenesis and tissue remodelling by the production of growth factors, cytokines, chemokines and matrix metalloproteinases.
- cyclooxygenase-2 upregulation.
- Suppression of antitumour adaptive immune responses.

Mechanisms by which adaptive immune cells modulate cancer

Direct mechanisms

- Inhibition of tumour growth by antitumour cytotoxic-T-cell activity.
- Inhibition of tumour growth by cytokine-mediated lysis of tumour cells.

Indirect mechanisms

- Promotion of tumour growth by regulatory T cells that suppress antitumour T-cell responses.
- Promotion of tumour development by humoral immune responses that increase chronic inflammation in the tumour microenvironment.

*In particular, tumour-infiltrating macrophages, mast cells and granulocytes.

Chronic inflammation and cancer development

When tissue homeostasis is chronically perturbed, interactions between innate and adaptive immune cells can be disturbed. Although duration and resolution are defining features of chronic versus acute inflammation, the cellular profiles, soluble mediators and downstream tissue-responsive pathways of the two states are also distinct (BOXES 1,2). The destructive cycles that are initiated within tissues by failure to appropriately engage and/or disengage either arm of the immune system can result in excessive tissue remodelling, loss of tissue architecture due to tissue destruction, protein and DNA alterations due to oxidative stress, and, under some circumstances, increased risk of cancer development.

Box 2 | **Chronic inflammation and disease pathogenesis**

What molecular mechanisms underlie harmful, excessive stimulation of immune-cell responses? Genetic predisposition underlies some disorders, such as pancreatitis, ulcerative colitis and some rheumatoid diseases. Others are associated with infectious pathogens that are able to evade natural tissue immune clearance mechanisms[96]. For example, *Helicobacter pylori*, a gram-negative bacterium, causes chronic gastritis in infected hosts, whereas infection with hepatitis B or hepatitis C virus (HBV and HCV, respectively) is linked to chronic hepatitis[97,98]. Unresolved inflammation also results from exposure to toxic factors such as asbestos or smoke, as well as from ongoing chemical or physical irritation, such as acid-reflux disease or exposure to ultraviolet (UV) light. Mutations and/or genetic polymorphisms in crucial genes that regulate cytokine function, metabolism and leukocyte survival have also been implicated as aetiological factors in chronic inflammation[99].

During acute inflammation, innate immune cells form the first line of immune defence and regulate activation of adaptive immune responses. By contrast, during chronic inflammation, these roles can be reversed — adaptive immune responses can cause ongoing and excessive activation of innate immune cells[78]. In arthritis, for example, activation of T and B lymphocytes results in antibody deposition into affected joints, prompting recruitment of innate immune cells into tissue[79]. Once within the tissue, activation and/or degranulation of mast cells, granulocytes and macrophages, in combination with humoral immune responses, leads to joint destruction[79]. By contrast, whereas acutely activated innate immune cells contribute to efficient T-cell activation, chronically activated innate immune cells can cause T-cell dysfunction through the production of reactive oxygen[100].

Regardless of the underlying initiating cause, if an infectious or assaulting agent is inadequately cleared and persists in tissue, or a tissue is subjected to ongoing insult and damage that fails to heal in a timely manner, host inflammatory responses can persist and exacerbate chronic tissue damage, which can cause primary organ dysfunction and systemic complications.

The association between immune cells and cancer has been known for over a century[5]. Initially, it was believed that leukocytic infiltrates, in and around developing neoplasms (FIG. 1), represented an attempt by the host to eradicate neoplastic cells. Indeed, extensive infiltration of NK cells in human gastric or colorectal carcinoma is associated with a favourable prognosis[6,7]. On the other hand, malignant tissues that contain infiltrates of other innate-immune cell types, such as macrophages in human breast carcinoma and mast cells in human lung adenocarcinoma and melanoma, tend to be associated with an unfavourable clinical prognosis[8–11]. Moreover, population-based studies reveal that individuals who are prone to chronic inflammatory diseases have an increased risk of cancer development[12]. In addition, over 15% of all human cancers are believed to be caused by infectious conditions[13], of which some — for example, chronic infection with *cag*$^+$ strains of *Helicobacter pylori* or with hepatitis viruses — indirectly promote carcinogenesis through induction of chronic inflammatory states[4].

Though seemingly contradictory, it was recently reported that cumulative antibiotic usage is associated with increased risk of breast cancer[14]. Do these data imply that bacterial infections are protective against breast cancer, or that antibiotic therapy is somehow deleterious? It is more likely that individuals who require frequent antibiotic regimens are at greater risk of cancer, either because they are maintaining low-level chronic inflammation as a consequence of defects in their natural immune defence mechanisms, and/or because they fail to normalize their immune status following infection. Some support for this hypothesis comes from experimental animal models in which immune-competent mice that lack key mediators of host immune defence, such as γ-interferon (IFNγ) and granulocyte-macrophage colony-stimulating factor (GMCSF), spontaneously develop various types of cancer in tissues that exhibit low-level chronic inflammation[15] (see Supplementary information S1 (table)).

One prediction that can be made from these population-based and experimental studies is that mutations or polymorphisms in genes that encode immune modifiers exist in individuals with chronic inflammatory disorders who have an increased risk of cancer. This is in fact the case — genetic polymorphisms in genes that encode crucial cytokines, proteases and signal-transduction proteins have been identified as aetiological factors in several chronic inflammatory disorders[12], indicating that therapeutics that are aimed at normalizing immune balance might be efficacious chemopreventatives. Clinical studies in which immune balance was restored in patients with active Crohn disease by treatment with GMCSF indicate that disease severity can be reduced by this approach[16].

Perhaps the most compelling clinical evidence for a causative link between chronic inflammation and cancer development comes from epidemiological studies reporting that inhibiting chronic inflammation in patients with pre-malignant disease, or who are predisposed to cancer development, has chemopreventative potential. These studies revealed that long-term usage of anti-inflammatory drugs, such as aspirin and selective cyclooxygenase-2 (COX2) inhibitors, significantly reduces cancer risk[17], indicating that COX2 or other key molecules that are involved in prostaglandin biosynthesis might be effective anticancer targets.

Given that the immune system is designed to eradicate 'damaged' cells or tissues, why does inflammation potentiate cancer development rather than protect against it? One plausible explanation for why tumour cells escape immune-surveillance mechanisms is that neoplastic microenvironments favour polarized chronic pro-tumorigenic inflammatory states rather than ones that represent acute antitumour immune responses[12,18]. Clinical data indicate that the 'immune status' of healthy individuals is distinct from that of those who harbour malignant tumours; in the latter, T lymphocytes are functionally impaired[19]. In addition, accumulations of chronically activated myeloid suppressor cells and

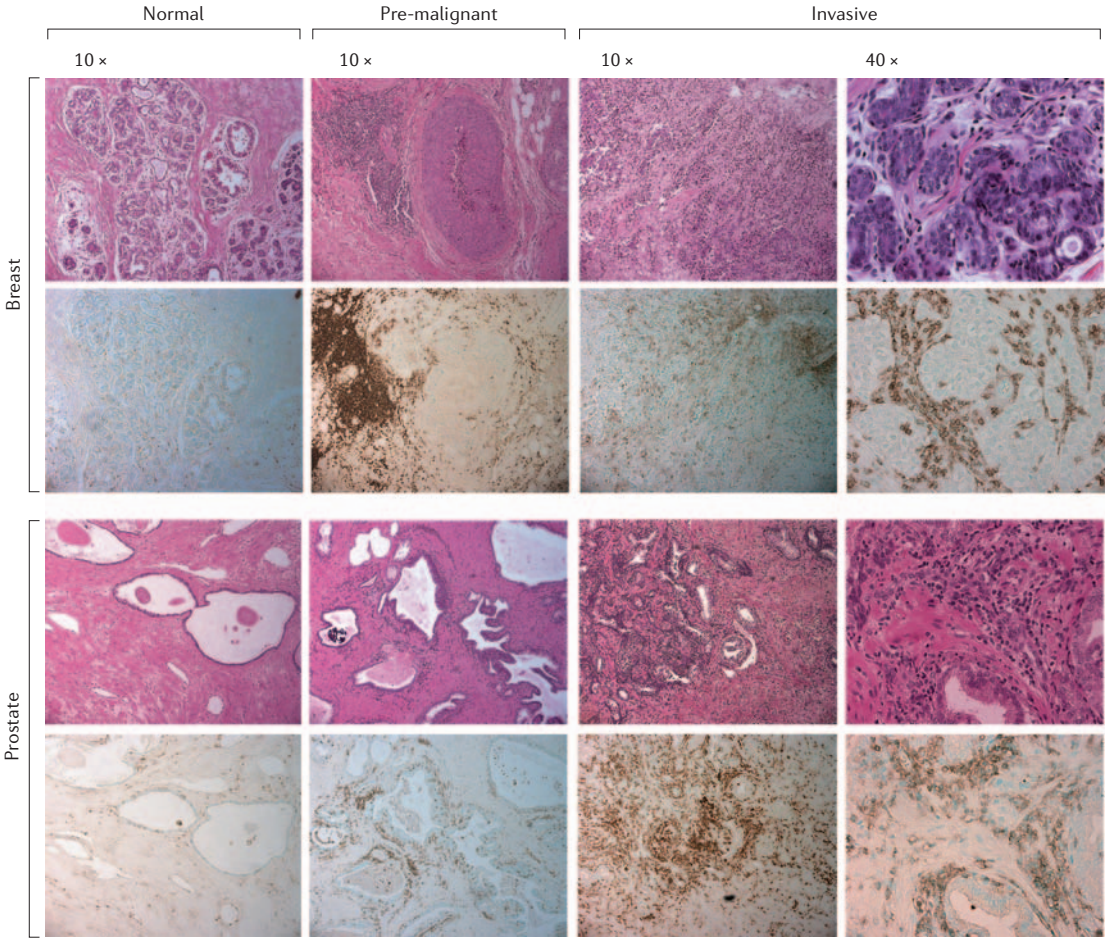

Figure 1 | **Inflammation in human breast and prostate cancer.** Many types of human carcinomas are characterized by abundant infiltrations of immune cells that are not revealed by standard histochemical analyses. Representative sections of normal, pre-malignant and malignant breast and prostate tissues that are stained with haematoxylin and eosin are shown (upper panels of each pair). When adjacent tissue sections are assessed for $CD45^+$ leukocytes (lower, brown stained panels), the extent of immune-cell infiltration into pre-malignant and malignant stroma is revealed.

regulatory T cells are found in the circulation, lymphoid organs and neoplastic tissues[20,21]. Together, immune states such as these can disable tumour-killing $CD8^+$ CTL responses and enable states of immune privilege that foster escape from antitumour immunity while simultaneously exploiting activated immune cells that, as we are beginning to appreciate, enhance cancer development.

Chronic inflammation in mouse models of cancer

In order to mechanistically evaluate tumour-promoting and antitumour roles for immune cells during cancer development, and to identify candidates to target for chemoprevention, several laboratories have experimentally manipulated and/or evaluated distinct immune-cell populations, and/or immune modulators, at discrete stages of cancer development in mouse models of *de novo* or spontaneous carcinogenesis (TABLE 1; see Supplementary information S1 (table)). We, and others, have utilized a mouse model of squamous epithelial carcinogenesis that is initiated by expression of oncogenes from human papillomavirus type 16 (HPV16) in mitotically active basal keratinocytes of the skin and the cervix[22,23] (TABLE 1).

HPV16 mice develop squamous epithelial pathologies that progress through distinctive histopathological stages (hyperplasia, dysplasia and carcinoma) that are similar to those found in individuals infected by HPV16 in the cervical epithelium[24]. Like epithelial carcinogenesis in humans, pre-malignant skin and cervix in HPV16 mice is characterized by chronic infiltration of innate immune cells in the stromal tissue[25,26] (FIG. 2). Interestingly, the profile of infiltrating inflammatory cells in skin is distinct from that in cervix — pre-malignant skin lesions contain, predominantly, infiltrating mast cells and granulocytes[27,28], whereas pre-malignant cervical lesions are characterized by infiltrating macrophages[26]. So, cancer development that is initiated by the expression of the same oncogenes, albeit in different tissue microenvironments, can result in distinct repertoires of infiltrating immune cells.

Do these infiltrating cells functionally contribute to cancer development? To address this question, we generated mast-cell-deficient/HPV16 mice and found attenuated neoplastic development, largely due to reduced activation of angiogenic vasculature and a

Regulatory T cells
T cells that can functionally suppress an immune response by influencing the activity of another cell type. Several phenotypically distinct regulatory-T-cell types might exist. The classic regulatory T cells are $CD4^+CD25^+$ $FOXP3^+$ T cells.

Table 1 | **Immunomodulation of cancer incidence in mouse models of *de novo* carcinogenesis**

Mouse cancer model	Target organ	Immune modulation	Result*	References
K14-HPV16	Skin	Mast-cell deficiency (*Kit*$^{W/WV}$)	Decreased keratinocyte proliferation; decreased angiogenesis	27
K14-HPV16	Skin	CD4$^+$ T-cell deficiency	Decreased CD11b$^+$ infiltration; decreased cancer incidence	71
K14-HPV16	Skin	CD8$^+$ T-cell deficiency	No effect	71
K14-HPV16	Skin	T- and B-cell deficiency (RAG1-deficient mice)	Decreased CD45$^+$ infiltration; decreased angiogenesis; decreased keratinocyte proliferation; decreased cancer incidence	28
K14-HPV16 and *Mmp9*-null	Skin	Transplantation with bone marrow cells that express MMP9	Increased keratinocyte proliferation; increased angiogenesis; increased cancer incidence	37
K14-HPV16/E2	Cervix	CD4$^+$T-cell deficiency	Increased cancer burden; increased cancer incidence;	72
K14-HPV16/E2	Cervix	*Mmp9*-null	Decreased angiogenesis; decreased cancer incidence	26
K14-HPV16/E2	Cervix	Bisphosphonate treatment	Decreased macrophage MMP9 expression; decreased angiogenesis; decreased cancer burden; decreased cancer incidence	26
RIP1-TAG2	Pancreas	*Mmp9*-null	Decreased angiogenesis; decreased cancer burden; decreased cancer incidence	40
MMTV-PyMT	Mammary gland	CSF1-null mutant mice (*Csf1*^{op}/*Csf1*^{op}); macrophage deficiency	Decreased late-stage mammary carcinoma; decreased pulmonary metastases	29
Apc$^{\Delta 716}$	Colon/small intestine	COX2 deficiency (*Ptgs2*-null mice)	Decreased cancer incidence; decreased cancer burden	47
Apc$^{\Delta 716}$	Colon/small intestine	COX2 inhibitor	Decreased tumour multiplicity; decreased tumour volume	46

*Results are reported as compared with transgenic littermate controls. *Apc*$^{\Delta 716}$, adenomatous polyposis coli Δ716; COX2, cyclooxygenase 2; CSF1, colony-stimulating factor 1; E2, 17β-estradiol; HPV16, human papillomavirus 16; K14, keratin 14; *Mmp9*, matrix metalloproteinase 9; MMTV, mouse mammary tumor virus; *Ptgs2*, prostaglandin endoperoxide synthase 2; PyMT, polyoma middle T antigen; RAG1, recombinase-activating gene 1; RIP1, rat insulin promoter 1; TAG2, simian-virus-40 large T antigen 2.

failure of keratinocytes to achieve hyperproliferative growth characteristics[27] (TABLE 1). This indicates that activation and/or degranulation of immune cells in neoplastic tissue upsets a crucial balance and thereby promotes cancer development. More significantly, studies such as these indicate that limiting or altering the presence of harmful innate immune cells in pre-malignant tissue minimizes oncogene-induced primary cancer development.

Are all tissue microenvironments susceptible to immune-cell-potentiated primary cancer development? Taking a similar approach, Lin and colleagues, using polyoma-middle-T-antigen (PyMT) transgenic mice as a model of mammary carcinogenesis, attenuated macrophage recruitment and found that failure to recruit macrophages into neoplastic tissue did not alter the hallmarks of pre-malignancy, but instead significantly delayed development of invasive carcinomas and reduced pulmonary metastasis formation[29] (TABLE 1). Metastatic potential was restored by transgenic expression of colony-stimulating factor 1 (CSF1) in mammary epithelium of CSF1-deficient/PyMT mice[29]. These experimental data, combined with the positive correlation in human cancers between CSF1 levels, macrophage recruitment and poor prognosis[30], indicate that macrophages are crucial for facilitating late-stage metastatic progression of tumours.

Other cells of the myeloid lineage have also been reported to contribute to tumour development[31]. However, some types of innate immune cells — in particular, NK cells — can protect against experimental tumour growth, in part by producing mediators with anti-angiogenic properties[32,33]. Together, these studies have induced a paradigm shift about the role of immune cells during malignant progression. Whereas the historical viewpoint was that host immunity is protective with regards to cancer, it is now clear that certain subsets of chronically activated innate immune cells promote growth and/or facilitate survival of neoplastic cells. Such an unexpected crucial role for innate immune cells as enhancers of tumour physiology raises questions about how they convey their tumour-promoting effects and whether, if understood, they can be harnessed to prevent or block immune-cell tumour-promoting properties while simultaneously activating antitumour immune responses?

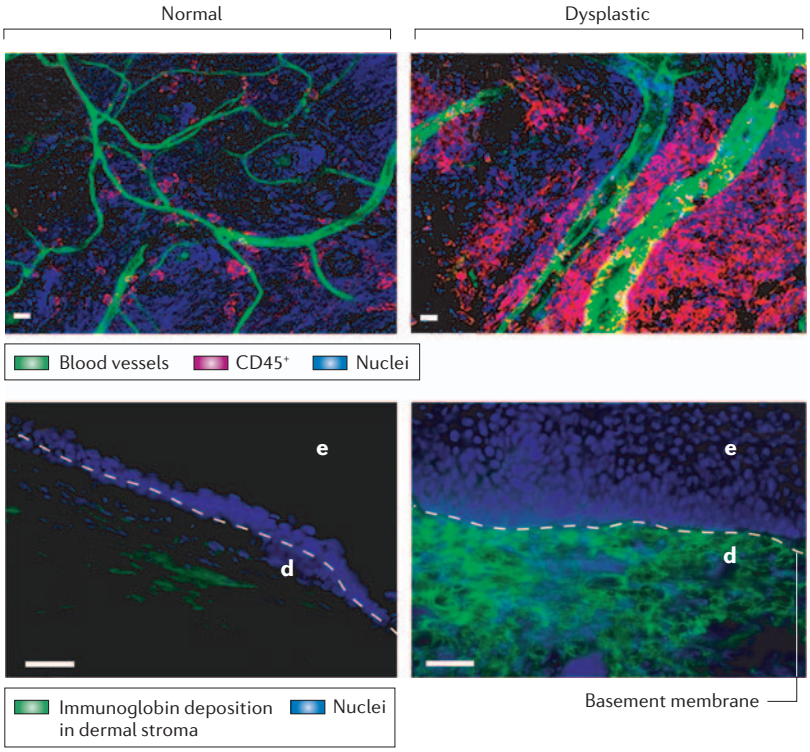

Figure 2 | **Inflammation and angiogenesis are hallmarks of squamous carcinogenesis in HPV16 transgenic mice.** Fluorescent angiography and immunohistochemical staining for CD45+ leukocytes in whole-tissue pieces (upper panels) shows parallel activation of blood vasculature (angiogenesis, shown in green) and immune-cell infiltration (red) of pre-malignant (dysplastic) skin tissue from HPV16 transgenic mice, compared with normal skin (cell nuclei are shown in blue; the scale bar represents 20μm). Interstitial immunoglobulin deposition (green) in the stroma of pre-malignant dysplastic skin from HPV16 transgenic mice, compared with normal skin, indicates robust humoral immune response during neoplastic progression (bottom panels; the scale bar represents 50μm). The dashed lines indicate the position of the epidermal basement membrane. d, dermis; e, epidermis.

Cancer development and innate immune cells

How then do chronically activated innate immune cells participate in cancer development? Which mechanisms and which inflammatory-cell-derived mediators are relevant for specific human malignancies — do these depend on organ, tumour stage or aetiology? Many of these questions remain unanswered; however, experimental models are beginning to elucidate molecular mechanisms by which innate immune cells regulate cancer processes (BOX 1). Because of their enormous plasticity and capacity to produce a myriad of cytokines, chemokines, metalloproteinases, ROS, histamine and other bioactive mediators, chronically activated innate immune cells are key modulators of cell survival (both proliferation and cell death) as well as regulators of ECM metabolism. Therefore, several physiological processes that are necessary for tumour development, such as increased cell survival, tissue remodelling, angiogenesis and suppression of antitumour adaptive immune responses, are regulated by leukocytic infiltrates in neoplastic environments. This is exemplified by a positive correlation between the number of innate immune cells (macrophages, mast cells and granulocytes) infiltrating human tumours and the number of blood vessels[34,35], and also by experimental findings in mouse models in which attenuating innate-immune-cell infiltration of pre-malignant tissue reduces angiogenesis and limits tumour development[27,28,31].

Matrix metalloproteinases. Numerous studies have documented increased expression of matrix metalloproteinases (MMPs) in human malignant tissue, often correlating with poor prognosis[36]. MMPs regulate tissue homeostasis and disease pathogenesis through pleiotropic biological effects, including remodelling of soluble and insoluble ECM components and cell–cell and cell–matrix adhesion molecules, that together alter crucial intracellular signalling pathways[36]. In both human and mouse models of cancer development, although some MMPs are produced by epithelial cells, the major source of MMPs is activated stromal cells — for example, fibroblasts, vascular cells and in particular, innate immune cells[36].

During skin and cervical carcinogenesis in HPV16 mice, MMP9 has been identified as a crucial immune-cell-derived mediator because of its ability to regulate epithelial proliferation, angiogenesis and overall cancer development[26,37]. Although amino-bisphosphonate-mediated blockade of MMP9 production by macrophages and genetic elimination of MMP9 significantly reduce cancer development in HPV16 mice (TABLE 1), infiltration of neoplastic tissue by immune cells is unperturbed by MMP9 absence[25,26]. This indicates that one mechanism by which inflammation potentiates cancer risk is the local delivery of MMP9.

Other experimental mouse models of cancer development have similarly identified MMP9 as a key inflammatory-cell-derived mediator of tumour-associated angiogenesis[38–40]. During pancreatic-islet carcinogenesis, for example, Bergers and colleagues determined that MMP9, which is produced predominantly by macrophages, regulates angiogenesis by mobilizing ECM-sequestered vascular endothelial growth factor (VEGF) and stimulating vascular endothelial cell proliferation and subsequent angiogenesis[40] (TABLE 1). The processing of pro-growth factors is not a unique property of MMP9 — in fact, several MMP family members are known to possess this property, and some of them also regulate acute inflammation through their ability to process chemokines[41]. MMP7 that is produced by osteoclasts has emerged as a significant regulator of prostate-cancer bone metastases by virtue of its ability to process RANKL (receptor-activator-of-nuclear-factor-κB ligand) and induce osteolysis[42].

As osteoclasts and macrophages are similarly derived from monocyte precursors, it will be interesting to determine if bisphosphonate therapy attenuates MMP7 production by osteoclasts in the same way that it inhibits macrophage MMP9 production during cervical carcinogenesis[26]. Bisphosphonates are known to significantly reduce the incidence of skeletal-related events during breast-cancer metastases to bone[43]. Therefore, perhaps the mechanisms by which this is achieved are monocyte blockade of MMP production and subsequent inhibition of the skeletal complications that result from bone metastases.

COX2. Epidemiological studies have revealed that long-term usage of non-steroidal anti-inflammatory drugs (NSAIDs) reduces cancer risk[17,44]. This is probably due to their inhibition of COX2, which is a multifunctional enzyme that is involved in prostaglandin biosynthesis, the expression of which is upregulated in inflamed and neoplastic tissues[17]. In several human epithelial cancers, expression of COX2 correlates with poor prognosis, and pharmacological inhibition of COX2 reduces cancer incidence[17]. Similar results have been found in rodent models of cancer development — in the mammary gland, COX2 overexpression induces carcinogenesis[45], whereas pharmacological inhibition and/or genetic deletion of COX2 reduces cancer development[46,47] (see Supplementary information S1 (table)).

Stromal cells — in particular, immune cells — as well as neoplastic cells are known to upregulate COX2 expression during malignant progression. Therefore, the efficacy of COX2-inhibitor-based therapies might be achieved by the regulation or the normalization of distinct biochemical and/or signalling pathways that are unique to each individual cell type[48,49]. COX2 is believed to exert its tumour-promoting effects by increasing cell survival and regulating signalling pathways that are involved in angiogenesis, inflammation and immune surveillance. However, the crucial molecules that mediate these effects remain largely undefined, though they might include the prostaglandin E_2 receptor EP2 subtype (PTGER2)[50].

Pro-inflammatory cytokines. Tumour microenvironments are also rich in immune-cell-derived cytokines, chemokines and pro-angiogenic mediators — for example, tumour necrosis factor-α (TNFα), transforming growth factor-β (TGFβ), VEGF, and interleukins 1 (IL-1) and 6 (IL-6)[12]. Production of VEGF is one mechanism by which tumour-infiltrating leukocytes increase angiogenesis and foster tumour development[34,51]. Similarly, TNFα, a key cytokine that is mobilized during acute inflammation, mediates cancer development[52]. Mice that are deficient for either TNFα or TNFα receptors have reduced susceptibility to chemically induced skin cancers and develop fewer experimental metastases (see Supplementary information S1 (table)). As TNFα receptors are expressed on both epithelial and stromal cells, TNFα facilitates cancer development directly, by regulating the proliferation and survival of neoplastic cells, as well as indirectly, by exerting its effects on endothelial cells, fibroblasts and immune cells in tumour microenvironments[12]. Clinical trials are currently underway to assess the efficacy of TNFα antagonists in patients with breast and ovarian cancer[52,53].

Recently, a functional link was elucidated between TNFα and the pro-inflammatory transcription factor nuclear factor κB (NFκB), revealing the complexity of paracrine signalling mechanisms between innate immune cells and evolving neoplastic cells. Using a mouse model of cholestatic hepatitis that predisposes mice to hepatocellular carcinoma, Pikarsky and colleagues reported that hepatocyte survival and malignant progression are regulated by NFκB, the activation state and cellular localization of which was under paracrine TNFα control[54]. Inhibition of TNFα or induction of the super-repressor mutant of IκB (inhibitor of NFκB) in transgenic animals during the later stages of neoplastic progression resulted in failure to progress to hepatocellular carcinoma[54]. This indicates that TNFα, which is produced by surrounding parenchyma, activates an NFκB-dependent anti-apoptotic pathway during the time at which the foci of pre-malignant hepatocytes develop into tumours.

Karin and colleagues came to a similar conclusion using a mouse model of colitis-associated cancer[55]. However, in these studies deletion of the inhibitor of NFκB kinase (IKKβ) — a key intermediary of NFκB — in intestinal epithelial cells did not decrease intestinal inflammation, as measured by inflammatory cytokine production, but instead reduced susceptibility to inflammation-induced intestinal tumours[55]. Moreover, specific deletion of IKKβ in myeloid cells resulted in formation of smaller inflammation-associated colon cancers and correlated with reduced production of tumour-promoting paracrine factors, including TNFα[55].

An important feature of these studies was that NFκB activation was selectively ablated in different cellular compartments of the developing tumour masses and/or at different stages of tumour development (see Supplementary information S1 (table)). This revealed that the NFκB pathway has a dual role in tumour promotion — first, by preventing apoptosis of cells with malignant potential, and second, by stimulating production of pro-inflammatory cytokines by cells of myeloid origin in tumours. These pro-inflammatory cytokines then contribute in a paracrine fashion to neoplastic cell proliferation and increase survival of initiated, and/or otherwise 'damaged', epithelial cells. These elegant approaches offer novel insights into differential regulation of pre-malignant and malignant states by inflammation, and the complexities and downstream activities of NFκB in distinct cellular compartments.

Antitumour adaptive immunity. Chronically activated innate immune cells can also indirectly contribute to cancer development through suppression of antitumour adaptive immune responses, allowing tumour escape from immune surveillance. A subset of innate immune cells (for example, myeloid suppressor GR^+CD11b^+ cells) accumulate in tumours and lymphoid organs[18,21,56]. Myeloid suppressor cells are known to induce T-lymphocyte dysfunction by direct cell–cell contact and by production of immunosuppressive mediators, and therefore actively inhibit antitumour adaptive immunity[21,56]. In addition, malignant lesions attract regulatory T cells that are also known to suppress effector functions of cytotoxic T cells[18]. Classic regulatory T cells are $CD4^+CD25^+FOXP3^+$; however, different subtypes might also exist. Initial investigations have revealed that *in vivo* depletion of regulatory T cells using antibodies against CD25 enhance antitumour T-cell responses and induce regression of experimental tumours[57,58]. An elegant study by Curiel and colleagues revealed that tumour-derived macrophages from patients with ovarian cancer produce CCL22, a chemokine that mediates trafficking of regulatory T cells to tumours[20].

These regulatory T cells in patients with ovarian cancer suppressed tumour-specific T-cell immunity, and their presence correlated with reduced survival. Therefore, in the vicinity of a growing neoplasm, the balance between innate and adaptive immunity is often disturbed in favour of cancer progression.

Different tissue, different target. Many types of chronically activated immune cells therefore exert tumour-promoting effects directly by influencing proliferation and survival of neoplastic cells, as well as by indirectly modulating neoplastic microenvironments to favour tumour progression (BOX 1). How can these diverse mechanisms be translated into the development of broadly applicable therapeutical approaches? Should future anticancer strategies focus on regulating COX2 activity, NFκB activation, TNFα bioavailability or crucial extracellular protease actvity? When addressing these questions, it is important to remember that all organs are endowed with unique cell-death and damage-response pathways that typically invoke acute activation of innate immune cells. In skin, for example, terminal differentiation is the mode by which keratinocytes die, and in contrast to colitis-associated and hepatocellular carcinoma, inhibiting NFκB in skin keratinocytes promotes epidermal hyperplasia and the development of spontaneous squamous cell carcinomas[59,60]

On the other hand, blockade of TNFα attenuates skin-tumour formation[61]. Therapeutically regulating multifunctional immunomodulators such as NFκB, TNFα, COX2 or MMPs requires careful risk assessments as systemic inhibition might have unfavourable outcomes, which are the result of cell-type and environment-dependent activities that differentially regulate tissue homeostasis. If systemic modulation can be tolerated without adverse side-effects, combining immunomodulating cytostatic therapies with radiation or cytotoxic drugs might be beneficial. Some success has been demonstrated with this approach both in cell lines, where overexpression of a 'super-repressor' of NFκB enhanced the activity of cytotoxic drugs[62], and in the clinic, where proteasome inhibitors increased the efficacy of chemotherapy in some patients[63].

Adaptive immunity and cancer

Whereas it has become generally accepted that chronic activation of innate immune cells contributes to cancer development, the role of adaptive immune cells is still a matter of debate. This is perhaps best exemplified by epidemiological studies of cancer incidence in patients with either AIDS or organ transplants who have chronic suppression of their adaptive immune compartment (TABLE 2). In these two groups, the relative risk (RR) of cancer development varies considerably depending on organ site and cancer aetiology. It is well known that the RR for viral-associated cancers, in particular human-herpesvirus-8-associated Kaposi sarcoma, Epstein–Barr-virus-associated non-Hodgkin lymphoma and HPV-associated squamous carcinoma, are elevated in immune-suppressed individuals (TABLE 2), owing largely to the fact that chronic immune suppression fails to provide protection against viral infections or viral re-activation[64]. Overall, the RR for invasive malignancies, other than Kaposi sarcoma, non-Hodgkin lymphoma and non-melanoma squamous cancers, is approximately 2.5; however, the RR varies considerably between individual cancers.

Some cancer types occur with increased frequency in selected groups of immune-compromised patients for reasons that are unrelated to immune suppression. For example, chronic exposure to tobacco carcinogens is associated with an increased RR for cancers of the lung, lip, mouth and pharynx in AIDS patients[65]. Similarly, the RR of lung cancer, head and neck cancer, oesophageal cancer, gastrointestinal cancer and pancreatic cancer in patients who have had liver transplants is increased when there is a previous history of alcohol and tobacco use[66,67]. On the other hand, the RR for the most common non-viral-associated solid tumours of epithelial origin is decreased in immune-suppressed patients; some of these in fact have an RR less than 1.0 (TABLE 2). In particular, the RRs for breast, prostate and bladder cancer are significantly reduced in both patients who have had organ transplants and patients with AIDS.

Although it is not surprising that immune suppression in the adaptive compartment fails to provide protection from the development of viral-associated or carcinogen-induced tumours, it is difficult to explain why immune suppression correlates with a decreased RR for some non-viral-associated cancers. Mouse models of cancer have similarly revealed that tumour development in immune-suppressed rodents varies depending on cancer type and cancer aetiology (TABLE 1; see Supplementary information S1 (table)). Some studies have reported an increased susceptibility to chemically induced cancers in mice with defined immunological defects, whereas others have reported a higher incidence of spontaneous tumours in immunocompromised mice that varies between organs and/or depends on the presence of persistent bacterial infection (see Supplementary information S1 (table)).

These studies, together with the identification of tumour-associated antigens, have in part fuelled the development of antitumour immunotherapy approaches[68]. Although these approaches seem efficacious in principle, the reality is that, for well-established bulky tumours, activation of endogenous antitumour T-cell responses is often insufficient to induce full tumour regression[68]. Moreover, cancer vaccines have generally elicited low numbers of tumour-specific immune cells, and tumour-targeted T cells often fail to infiltrate solid tumours or they show a low avidity for tumour antigens[68,69]. Therefore, they suboptimally recognize target cells that express specific antigens. Furthermore, cancer vaccines must overcome the systemic immune suppression that is exerted by tumours. Some of these problems can be circumvented by immunotherapy approaches that make use of adoptive transfer, in which autologous anticancer T cells from the patient are generated and/or expanded *ex vivo* before being transferred back into the patient[70]. Therefore, despite the many challenges of cancer immunotherapy, it is clear that sufficient

Table 2 | **Human immune-deficient status and cancer risk**

Immune deficiency	Cohort size	Cancer type	Relative risk	References
AIDS-defining cancers/viral-associated cancers				
AIDS	122,993	Kaposi sarcoma	97.5 (male) 202.7 (female)	102
		Non-Hodgkin lymphoma	37.4 (male) 54.6 (female)	
		Skin (excluding Kaposi sarcoma)	20.9 (male) 7.5 (female)	
		Cervical	9.1	
AIDS	8,828	Kaposi sarcoma	545	103
		Non-Hodgkin lymphoma	24.6	
AIDS	302,834	Kaposi sarcoma	177.7	104
		Non-Hodgkin lymphoma	72.8	
		Cervical	5.2	
Liver transplant	187	Cutaneous	16.9	66
Liver transplant	174	Skin (non-melanoma)	70	105
Non-AIDS-defining cancers (with reduced RR)				
AIDS	302,834	Breast	0.5	104
		Prostate	0.5	
AIDS	122,993	Prostate	0.7	102
		Bladder	0.5	
		Breast	0.8 (HIV positive) 0.2 (post-AIDS onset)	
AIDS	8,828	Prostate	0.8	103
AIDS	62,157	Ovarian	0.58	106
		Breast	0.55	
		Uterine	0.28	
Kidney/heart transplant	25,914	Breast (year 1)	0.49	107
		Breast (year 2–11)	0.84	
Liver transplant	1,000	Breast, ovary, uterus and cervical	0.53	67
		Genitourinary	0.68	

numbers of adequately activated T lymphocytes can be beneficial for some patients. However, surpassing the hurdle of adequacy appears to be a difficulty.

Experimental rodent studies have also provided contradictory findings regarding the elimination of adaptive immune components and the activation of tumour-specific adaptive immune responses in cancer development (TABLE 1; see Supplementary information S1 (table)). These seemingly paradoxical statements are perhaps best exemplified by recent studies in which the functional significance of $CD4^+$ T lymphocytes was assessed during skin and cervical carcinogenesis in HPV16 mice[71,72]. Genetic elimination of $CD4^+$ T lymphocytes resulted in slightly delayed development of late-stage skin dysplasias and a modest reduction in skin cancer incidence[71]. By contrast, genetic elimination of $CD4^+$ T cells in female HPV16 mice that were undergoing oestrogen treatment to predispose them to cervical carcinogenesis resulted in a 10-fold increased tumour burden and a 20% increase in carcinoma incidence compared with oestrogen-treated HPV16 controls[71,72].

The adaptive immune system can also differentially regulate cancer development within the same epithelial microenvironment, as a function of varied initiation. For example, mice that are deficient for αβ T cells have an increased incidence of methylcholantrene (MCA)-induced carcinomas compared with mice that contain αβ T cells. However, when the same cohorts were treated with 7,12-dimethylbenz[a]anthracene (DMBA) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA), the αβ-T-cell-deficient mice had a reduced susceptibility to carcinoma development compared with the controls[73] (see Supplementary information S1 (table)).

Likewise, paradoxical roles for NK T cells have been reported during cancer development[74]. NK T cells are $CD3^+$ T cells that also express some NK-cell markers and recognize glycolipid ligands that are presented by the major-histocompatibility-complex class-I-like molecule, CD1d[75]. It has been reported that NK T cells are involved in natural host immunity against chemically induced sarcomas[32]. On the other hand, it has also been reported that NK T cells can downregulate tumour

αβ T cells
Lymphocytes that express T-cell receptors consisting of heterodimers of α and β chains. αβ T cells recognize antigens when they are presented in association with major histocompatibility molecules.

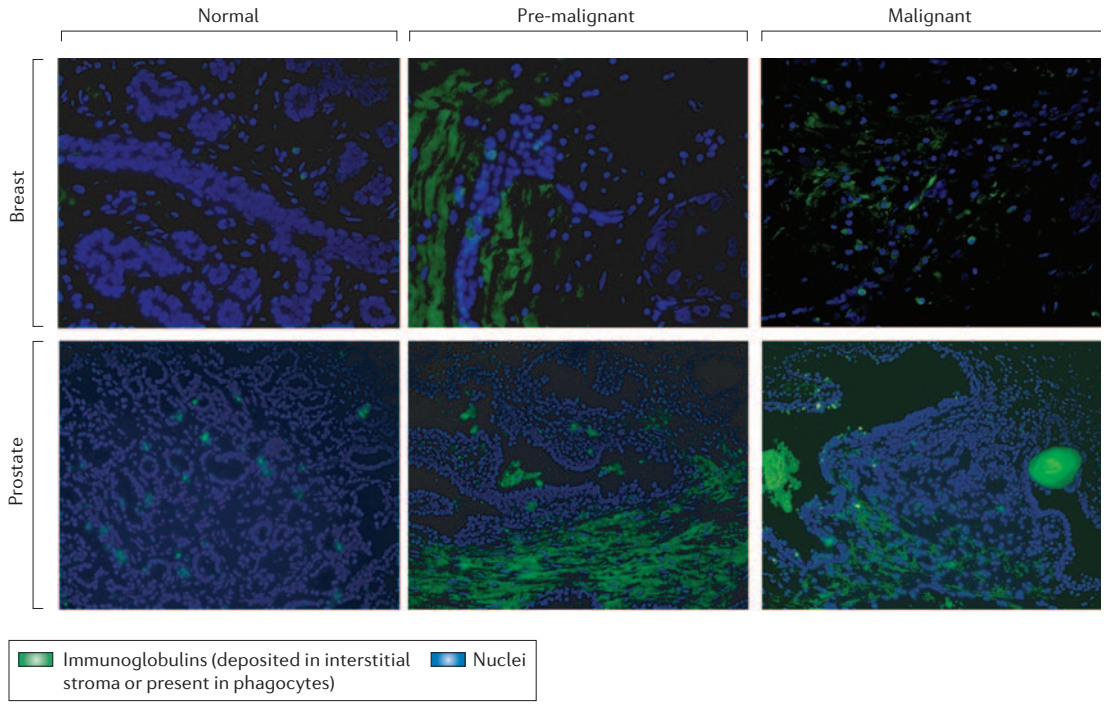

Figure 3 | **Humoral immune response in breast and prostate cancer.** Robust humoral immune responses are found during human breast and prostate tumorigenesis compared with normal tissue. The fluorescent images show that immunoglobulin deposits (green) are prominent in the interstitial stroma of pre-malignant and malignant prostate tissues (bottom panels). By contrast, during breast tumorigenesis (top panels), immunoglobulins are also found within phagocytes in pre-malignant and malignant tissues (shown by the colocalization of green fluorescence and the blue flourescence of the cell nuclei)[101].

Chronic idiopathic thrombocytopaenia
An autoimmune disease that involves autoantibody-mediated eradication of platelets, resulting in a reduced overall number of platelets. The primary clinical symptom is increased and prolonged bleeding.

Autoimmune haemolytic anaemia
An autoimmune disease that involves autoantibody-mediated premature destruction of erythrocytes, resulting in anaemia.

Systemic lupus erythematosus
A multi-system inflammatory disease that is characterized by autoantibody production and deposition of immune complexes in many organs, causing a broad spectrum of manifestations.

Fc receptors
A family of receptors that are involved in recognition of the Fc portion of antibodies. Fc receptors are expressed on the surface of various immune cells. Depending on the type of Fc receptor that is expressed, crosslinking can result in degranulation, activation of phagocytosis, and cytokine release.

Complement cascades
The complement system is made up of more than 25 components that are present in serum. Foreign antigens and immune complexes activate the complement activation cascade, resulting in formation of lytic membrane-attack complexes and liberation of potent pro-inflammatory factors.

immunosurveillance against transplanted tumours[76,77]. This paradoxical influence of NK T cells during cancer development is probably a consequence of their inherent capacity to produce both pro-inflammatory T-helper-1 cytokines and anti-inflammatory T-helper-2 cytokines[74]; therefore, the nature and balance of surrounding stimuli might determine which type of NK-T-cell-induced T-helper response dominates and contributes to malignant outcome.

In this way, rodent models parallel human cancer and indicate that the adaptive immune system differentially modulates *de novo* cancer development in an organ-dependent and aetiology-dependent manner. The paradoxical influence of the adaptive immune system during these processes raises many questions, the understanding of which is crucial if treatment modalities involve adaptive-immune-based therapies. Depending on the microenvironment or cancer aetiology, adaptive-immune-based therapies could either exacerbate or arrest neoplastic disease.

Adaptive immunity, inflammation and cancer

Recent advances in understanding underlying mechanisms of chronic autoimmune diseases have revealed that adaptive immunity has a crucial role in regulating and activating innate immune cells in affected tissues[78]. For example, interstitial immunoglobulin (Ig) deposition has been observed in tissues that are heavily infiltrated by innate immune cells in patients with rheumatoid arthritis[79]. B-lymphocyte depletion in these patients decreases disease severity, as it also does in chronic idiopathic thrombocytopaenia, autoimmune haemolytic anaemia and systemic lupus erythematosus[80]. This indicates that immunoglobulin deposition contributes to chronic inflammation and disease pathogenesis rather than merely correlating with it.

Antibodies that are deposited into interstitial tissues can trigger activation of innate immune cells by the cross-linking of Fc receptors or the activation of complement cascades[78]. As $CD4^+$ and $CD8^+$ T lymphocytes are important modulators of such tissue-damaging B-lymphocyte responses, and because Ig deposition is found in human pre-malignant and malignant tissues (FIG. 3), it is possible that imbalanced or altered adaptive-immune-cell interactions represent underlying mechanisms that regulate the onset and/or maintenance of chronic inflammation that is associated with cancer development.

To address this possibility, we evaluated the role of adaptive immune cells in HPV16 mice and found that combined B- and T-lymphocyte deficiency attenuated innate-immune-cell infiltration of pre-malignant skin[28]. As a consequence, blood vasculature remained quiescent, keratinocytes failed to attain a hyperproliferative phenotype and the overall incidence of invasive carcinomas decreased to ~6%, compared with ~50% in the controls[28]. Adoptive transfer of B lymphocytes or serum from HPV16 mice into B- and T-cell-deficient/HPV16 mice restored characteristic chronic inflammation in

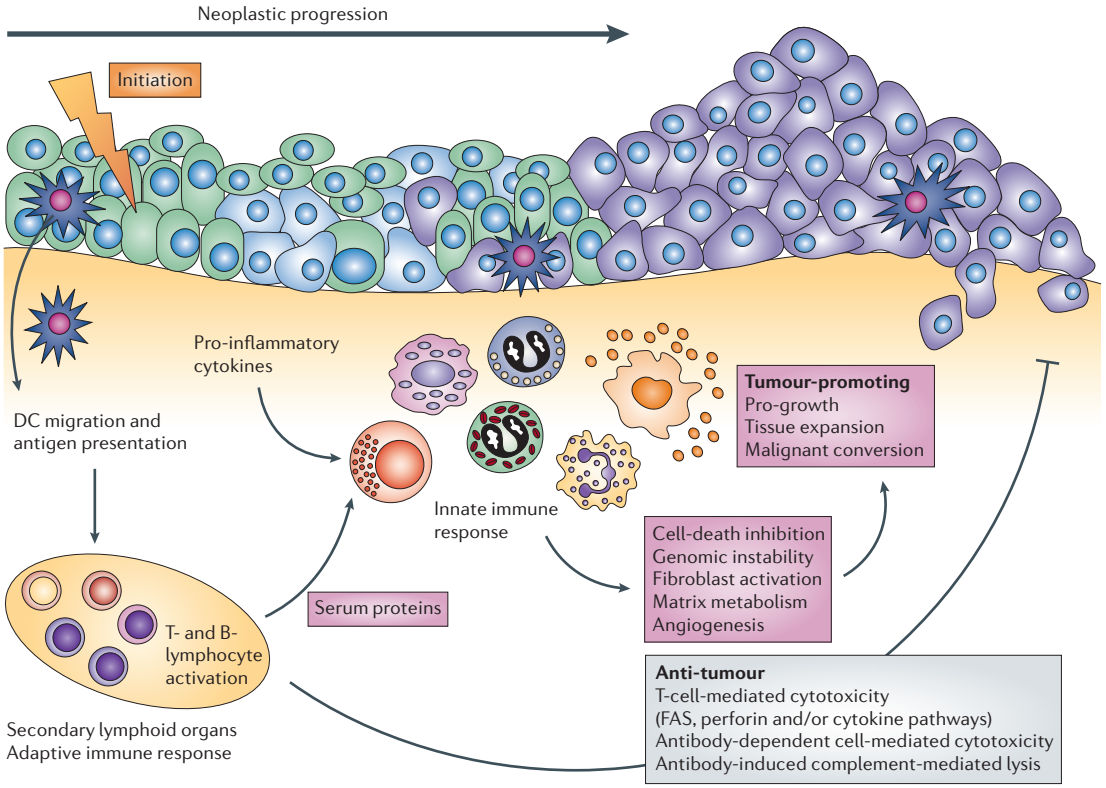

Figure 4 | **A model of innate and adaptive immune-cell function during inflammation-associated cancer development.** Antigens that are present in early neoplastic tissues are transported to lymphoid organs by dendritic cells (DCs) that activate adaptive immune responses resulting in both tumour-promoting and antitumour effects. The pathways that regulate DC trafficking during early cancer development and the exact nature of the antigen(s) remains to be established. Activation of B cells and humoral immune responses results in chronic activation of innate immune cells in neoplastic tissues. Activated innate immune cells, such as mast cells, granulocytes and macrophages, promote tumour development by the release of potent pro-survival soluble molecules that modulate gene-expression programmes in initiated neoplastic cells, culminating in altered cell-cycle progression and increased survival. Inflammatory cells positively influence tissue remodelling and development of the angiogenic vasculature by production of pro-angiogenic mediators and extracellular proteases. Tissues in which these pathways are chronically engaged exhibit an increased risk of tumour development. By contrast, activation of adaptive immunity also elicits antitumour responses through T-cell-mediated toxicity (by induction of FAS, perforin and/or cytokine pathways) in addition to antibody-dependent cell-mediated cytotoxicity and antibody-induced complement-mediated lysis.

pre-malignant skin and reinstated angiogenesis and keratinocyte hyperproliferation, which are parameters that are essential for full malignancy[28].

These data indicate that B lymphocytes, and factors that are present in serum, are essential for establishing chronic inflammatory states that are associated with pre-malignant progression (FIG. 2), and are therefore involved in enhancing the neoplastic pathways that are necessary for skin cancer development. B lymphocytes do not significantly infiltrate pre-malignant skin of HPV16 mice, indicating that priming of B lymphocytes occurs in draining lymph nodes by skin-derived antigen-presenting DCs. It is not yet clear which signals induce DC trafficking to draining lymph nodes. The prevailing model for DC migration from inflamed tissue to lymph nodes involves expression of chemokine (C-C motif) receptor 7 (CCR7) and specific integrins by DCs, and the existence of a chemotactic gradient from the periphery to lymphatic vessels[81]. However, whether similar pathways are involved during cancer progression is still unclear. In addition, it remains to be investigated which skin-derived antigens trigger tumour-promoting B-lymphocyte responses. Nor is it known whether these are antigens that are derived from the HPV16 early-region genes or antigens that are derived from stromal molecules undergoing remodelling in pre-malignant skin. Are these results a unique feature of skin carcinogenesis in HPV16 mice, or do other experimental models or clinical data support a crucial role for B lymphocytes during epithelial cancer development?

Potential tumour-promoting roles for B lymphocytes and/or antibodies were described over 50 years ago, albeit without elucidation of any underlying mechanisms. These early studies demonstrated that passive transfer of tumour-specific antibodies increased outgrowth of transplanted tumour cells and chemically induced tumours[82–85], whereas absence of B lymphocytes limited tumour formation[86,87]. More recently, it was reported that low antibody-responder mice were less susceptible to DMBA/TPA-induced

skin carcinogenesis as compared with high antibody-responder counterparts[88] (see Supplementary information S1 (table)), and that active immunization of cancer-prone immune-proficient mice resulted in tumour-specific antibody responses and increased carcinogenesis after chemical promotion[89]. Consistent with the link between B lymphocytes and the initiation of chronic inflammation in HPV16 mice, Barbera-Guillem and colleagues demonstrated that antitumour humoral immune responses increase outgrowth and invasion of murine and human tumour-cell xenografts through recruitment and activation of granulocytes and macrophages[90].

In the clinical arena, there is a vast literature that describes the occurrence of autoantibodies in the serum of cancer patients, and interstitial antibody deposition in human tumours[91] (FIG. 3). Moreover, the early presence of autoantibodies (in particular, antinuclear and smooth-muscle antibodies) in the serum correlates with an unfavourable prognosis[92]. Does this correlation indicate that individuals with tumours that progress harbour a higher antigen load and therefore trigger greater antibody production, or does it indicate that the presence of antibodies predisposes patients to development of more advanced or recurrent cancers? Although the answer is not clear, these data indicate that B lymphocytes are also involved in human cancer development and therefore necessitate a more mechanistic evaluation of their role and specificity to determine if they represent tractable targets for anticancer therapy.

Conclusion and Perspective

Clinical and experimental studies now indicate that innate and adaptive immune cells are significant, albeit sometimes paradoxical, determinants of epithelial tumorigenesis (FIG. 4). On the basis of classical theories of immune surveillance and more recent awareness of the tumour-promoting properties of innate immune cells, researchers are now investigating the efficacy of novel anticancer strategies that are based on immunotherapeutics that can either bolster antitumour adaptive immunity or neutralize cancer-promoting properties of innate immune cells.

An issue to be resolved with these powerful approaches is how therapeutically manipulating one arm of the immune system affects the anticancer or cancer-promoting properties of the other. For example, induction of anticancer humoral immune responses might be beneficial for patients with established cancer; however, given the observation that inflammation-associated epithelial carcinogenesis is B-lymphocyte-dependent[28], activation of humoral immune responses in patients who are predisposed to cancer development or in patients with latent or pre-malignant disease might enhance neoplastic programming of tissue rather than eradicating it. In these latter patients, it might be beneficial to monitor their serum for indications of B-cell activation and/or humoral immunity, as this might reveal a therapeutic window for anti-B-lymphocyte-based therapies or for modalities that are aimed at neutralizing tumour-promoting properties of innate immune cells.

In addition to NSAIDs and COX2 inhibitors having been shown to be efficacious[17], and clinical trials with GMCSF[16], TNFα antagonists[52,53] and adoptive transfer of autologous anticancer T cells[70] being encouraging, recent successes with B-lymphocyte depletion for relief of rheumatoid arthritis and systemic lupus erythematosus[93,94] have demonstrated safety and instill optimism for the approach. Although one or other of these host-targeted strategies might provide a therapeutic advantage, it seems reasonable to consider combinatorial immunomodulating strategies that target cancer-promoting properties of both innate and adaptive immune-cell populations while simultaneously exploiting their unique anticancer attributes without interfering with their normal functions.

Clinical use of protease and COX2 inhibitors as anticancer therapeutics has been minimized[44,95], largely because of the failure to take into account the crucial role that these powerful mediators have in maintaining and normalizing tissue homeostasis. Nevertheless, once risk assessments have been carried out, a prediction for these new host-targeted approaches is that immunomodulating therapies will be used to the patient's advantage and might offer the possibility of normalizing cancer-prone tissues prior to the appearance of bulky disease. Alternatively, if they are used in combination with standard antitumour approaches (for example, cytotoxic drugs), they could provide an overwhelming assault on malignant cancer cells. Therefore, the goal should be to determine optimal and tolerable combinations of immunomodulating and cytotoxic therapies that will result in significant survival and quality-of-life improvements for patients with cancer.

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Competing interests statement
The authors declare no competing financial interests.

DATABASES
The following terms in this article are linked online to:
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FURTHER INFORMATION
Lisa M. Coussens's homepage: http://cc.ucsf.edu/coussens/index.html

SUPPLEMENTARY INFORMATION
See online article: S1 (table)
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Supplementary Table 1. Immune modulation of spontaneous, chemically and bacterially-induced cancers

Immune modulator	Target Organ	Initiation	Result[a]	Ref
K5-COX-2	skin	DMBA	↑ carcinoma incidence	1
K5-COX-2	urinary bladder	spontaneous	↑ inflammation ↑ angiogenesis ↑ epithelial proliferation ↑ carcinoma incidence	2
MMTV-COX-2	mammary gland	spontaneous	↓ epithelial cell apoptosis ↑ adenocarcinoma incidence	3
K19-COX-2/mPGES-1	gastric mucosa	bacteria	↑ macrophage infiltration gastric mucosal hyperplasia	4
K19-COX-2/mPGES-1	gastric mucosa	antibiotic therapy	normal	4
Tnfα $^{-/-}$	skin	DMBA/TPA	↓ carcinoma incidence	5
Tnfα $^{-/-}$	skin	repetitive DMBA	↓ tumour multiplicity	5
TnfαR $^{-/-}$	skin	DMBA/TPA	↓ carcinoma incidence	6
TNFα antagonist	skin	DMBA/TPA	↓ carcinoma incidence	7
K14-IκBαM (DN)	skin	spontaneous	↑epidermal hyperplasia	8
K5-*IκBα*	skin	spontaneous	↑ inflammation ↑ cancer incidence	9,10
K5-*IκBα*	skin	TNFR1 $^{-/-}$	↓ inflammation ↓ cancer incidence	9,10
enterocyte IKKβ-deletion	colon	azoxymethane/dextran sulfate	↓ adenocarcinoma incidence	11
myeloid IKKβ-deletion	colon	azoxymethane/dextran sulfate	↓ inflammatory cytokines ↓ tumor burden	11
Mdr2 $^{-/-}$	liver	spontaneous	Non-suppurative inflammatory cholangitis ↑ metastatic hepatocellular carcinoma	12
Mdr2 $^{-/-}$ + hepatocyte-specific NFκB super-repressor	liver	spontaneous	↓ inflammation-associated hepatocellular carcinoma	13
Gmcsf $^{-/-}$/*Ifnγ* $^{-/-}$	systemic	bacteria	↑ spontaneous cancers	14
		antibiotic therapy	no cancer	14
Ifnγ-R $^{-/-}$	skin	MCA	↑ carcinoma incidence	15
Stat1 $^{-/-}$	skin	MCA	↑ carcinoma incidence	15
Trp53 $^{-/-}$/*IfnγR* $^{-/-}$	systemic	spontaneous	↑ carcinoma incidence	15
Pfp $^{-/-}$	systemic	spontaneous	↑ lymphoma incidence ↑ adenocarcinoma incidence	16
Ifnγ $^{-/-}$	systemic	spontaneous	↑ lymphoma incidence ↑ adenocarcinoma incidence	16
Pfp $^{-/-}$/ *Ifnγ* $^{-/-}$	systemic	spontaneous	↑↑ adenocarcinoma incidence	16
Pfp $^{-/-}$	skin	MCA	↑ carcinoma incidence	17
Pfp $^{-/-}$	skin	DMBA/TPA	no change in cancer incidence	17
Pfp $^{-/-}$	muscle	MSV infection	↓ regression of cancer	17
NK-T cell-deficiency	skin	MCA	↑ carcinoma incidence	18,19
NK cell-deficiency	skin	MCA	↑ carcinoma incidence	18,19
Rag2 $^{-/-}$	skin	MCA	↑ carcinoma incidence	20
Rag2 $^{-/-}$/*Stat1* $^{-/-}$	systemic	spontaneous	↑ carcinoma incidence	20

TCRδ-deficiency	skin	MCA	↑ cancer incidence	21
TCRδ-deficiency	skin	DMBA/TPA	↑ cancer incidence	21
TCRβ-deficiency	skin	MCA	↑ cancer incidence	21
TCRβ-deficiency	skin	DMBA/TPA	↓ cancer incidence	21
cKit$^{W/WV}$	colon	1,2-dimethylhydrazine	mast cell-deficiency ↓ adenocarcinoma incidence	22
L-Biozzi mice (low antibody response) vs. H-Biozzi mice (high antibody response)	skin	DMBA/TPA or DMBA	↓ tumour incidence in L-mice vs H-mice	23

a, results reported as compared to wildtype littermate controls

COX, cyclooxygenase; DMBA, 7,12-dimethylbenz[a]anthracene; DN, dominant negative; GM-CSF, granulocyte-macrophage colony stimulating factor. IκB, inhibitor kappa B; Ifn, interferon; K5, keratin 5; K14, keratin 14; K19, keratin 19; MCA, methylcholantrene; MSV, Moloney sarcoma virus; NK, natural killer; NF-κB, nuclear factor kappa B; Pfp, perforin; mPGES-1, microsomal prostaglandin E synthase; Rag, recombinase activating gene; TCR, T cell receptor; TNFα, tumour necrosis factor-alpha; TPA, 12-O-tetradecanoylphorbol-13-acetate

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