Infection and Cancer: Revaluation of the Hygiene Hypothesis

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Abstract

Several studies have shown that persistent infections and inflammation can favour carcinogenesis. At the same time, certain types of pathogens and anti-tumour immune responses can decrease the risk of tumourigenesis or lead to cancer regression. Infectious agents and their products can orchestrate a wide range of host immune responses, through which they may positively or negatively modulate cancer development and/or progression. The factors that direct this dichotomous influence of infection-mediated immunity on carcinogenesis are not well understood. Even though not universal, several previous reports have investigated the inverse link of pathogen-induced “benign” inflammation to carcinogenesis and various other pathologies, ranging from autoimmune diseases to allergy and cancer. Several models and ideas are discussed in this review, including the impact of decreased exposure to pathogens, as well as the influence of pathogen load, the timing of infection and the type of instigated immune response on carcinogenesis. These phenomena should guide future investigations into identifying novel targets within the microbial and host proteome, which will assist in the development of cancer therapeutics and vaccine remedies, analogous to earlier efforts based on helminthic components for the prevention and/or treatment of several pathologies.
Abbreviations: BCG, Bacillus Calmette-Guérin; CD20, B-lymphocyte antigen CD20; CDK, cyclin-dependent kinase; CTL, cytotoxic T lymphocytes or cytotoxic T cells; CTLA-4, cytotoxic T lymphocyte-associated antigen 4; FDA, Food and Drug Administration; HER2, human epidermal growth factor receptor 2; HIF-1α, hypoxia-inducible factor 1-alpha; HPV-E7, human papilloma virus protein E7; IgA, immunoglobulin A; IgM, immunoglobulin M; IL-10, interleukin 10; LPS, lipopolysaccharide; MAGE, melanoma-associated antigens; MDSC, myeloid-derived suppressor cells; MMP, matrix metalloproteinases; NK cells, natural killer cells; RB, retinoblastoma; T, Thomsen-Friedenreich mucin-type carbohydrate antigen; Th cells, T helper cells; TLR, Toll-like receptors; Tn, precursor of Thomsen-Friedenreich antigen; TNFα, tumor necrosis factor-alpha; Treg, regulatory T cells; VEGF, vascular endothelial growth factor.
Introduction

Even though the course of carcinogenesis is undoubtedly multifactorial, major attention has been attracted on the role of infectious diseases and the immune system in cancer development [1-4]. Several types of carcinomas are related to infections [2, 5], while inflammation is recognized as one of the hallmarks of cancer [1, 6] and inclusion of immunological assessments in cancer classification and prognosis, has been suggested [7, 8]. In contrast, immune responses, including those triggered by microorganisms, are known to decrease cancer risk or lead to tumour regression. The relationship between infection and tumourigenesis is not well understood and both favourable and unfavourable immune-mediated or direct anti-carcinogenic microbial effects have been observed. This review aims to provide an update primarily on the inverse association between infections and cancer and provide clues for potential underlying mechanisms. Attention is drawn to the hygiene hypothesis that attempts to explain the increased incidence of pathologies such as allergies, autoimmune diseases and cancer in the industrial world. Several historical observations and other theories, such as hormesis [9] and concomitant immunity [10], are revisited in order to lend more credence to the hygiene hypothesis.

The cancer hygiene hypothesis

Several decades ago, the hygiene hypothesis, referring to the lack of exposure to microbes at childhood, was introduced to explain the higher numbers of allergic and autoimmune diseases in the western world and urbanized communities [11-14]. More recently, the hygiene hypothesis has been re-stated to account for the association between microorganisms and cancer [13]. Following the same pattern observed with some immune pathologies, there is growing evidence of an increased cancer incidence in westernized economically-developed countries [15].
Socioeconomic status was also inversely associated with Hodgkin’s lymphoma [16], and daycare attendance was associated with a lower risk of acute lymphoblastic leukemia [17, 18]. The resemblance of the hygiene-immunopathology relationship to the one exhibited by hygiene and cancer is not surprising, given that preliminary observations have associated tumourigenesis with chronic immune-mediated disorders (Table 1); for example, an increased risk of cancer has been observed in autoimmune disease patients [19, 20], chronic allergic disorders have been connected to pro- and anti-tumour effects [21-24], and allergic cancer patients have been suggested to exhibit higher cure rates and more favourable disease progression [25]. Some experimental evidence may also support the cancer hygiene hypothesis, i.e. the anti-tumourigenic role of several inflammatory components, the ability of some commensals and benign gastrointestinal parasites like helminths, to downregulate inflammation, as well as the ability of pathogens and their products to stimulate anti-cancer immunity (see sections below). However, the hygiene hypothesis, as it stands, cannot rationalize why specific infectious agents (e.g. *H. pylori*; [26, 27]) or microbial products (e.g. lipopolysaccharide, LPS; [28, 29]) can exhibit both pro- and anti-carcinogenic functions and, therefore, many questions remain unresolved.

**Immune responses to infection and cancer**

Host immune response to pathogens generally involves non-specific effectors pre-existing locally in mucus (e.g. IgA, anti-microbial peptides, lysozyme) or plasma (natural IgM, complement), followed by activation of more specialized innate (e.g. macrophages, granulocytes, dendritic, mast, NK cells) and adaptive (T cells, B cells) immune processes, to facilitate clearance of pathogens or reduction of their impact [30]. Innate immune cell activation can trigger phagocytosis, release of antimicrobial compounds and pro-inflammatory cytokines, as well as lead to immune suppression, fibrosis, angiogenesis and wound healing [31]. T cells,
following pathogen recognition and depending on the antigen and local environment, develop into cytotoxic T cells (CTL) or T helper cells (Th), namely Th1, Th17 or Th2 cells, mediating different cytokine expression patterns, known as classical (Th1, Th17) or alternative (Th2) inflammation [32]. Th cells also stimulate production of antibodies from antigen-activated B cells. Another distinct cell subtype, regulatory T cells (Treg), particularly observed in chronic parasitic infections (e.g. helminths), have a role in preventing immune-mediated damage [33, 34]. Notably, the immune response pattern can vary during the infection course; in helminth infections, a Th1 to Th2 shift is commonly observed in parallel with infection progression, and may also signal the reduced effectiveness of a drug therapy [35, 36].

The various immune processes induced during infection may also be implicated in cancer. In 1863, it was Rudolf Virchow, who demonstrated the presence of leukocytes in neoplastic tissue (reviewed in [37]). Paul Ehrlich later suggested that the immune system continuously destroys spontaneously arising tumours (immune surveillance hypothesis), work that was updated by the cancer immunoediting hypothesis, stating that the immune system has a significant role in recognizing the antigenic properties of an emerging tumour [38, 39]. Both innate and adaptive immune cells are now known to localize at tumour sites, with specific cell subsets, densities and intra-tumour locations being associated with cancer risk or survival [8]. Antibodies against tumour-associated antigens have also been detected in cancer patients sera (International SEREX Program, The Ludwig Institute for Cancer Research). However, while several studies have considered the role of immunity in cancer survival/progression, the idea that an existing infection may further modulate the pro/anti-tumourigenic immune effect has been overlooked.
Infection as a carcinogenic factor

Some infectious agents can directly influence carcinogenesis; for instance, human papilloma virus protein E7 (HPV-E7) can bind the retinoblastoma (RB) tumour suppressor and the CDK inhibitor p21 in infected cells, promoting DNA replication and cell proliferation [40], while Hepatitis B virus can induce HIF-1α, stimulating angiogenesis [41]. Pathogens may also promote tumourigenesis indirectly (Table 1) [3, 4], by activating cancer-mediating host inflammatory pathways. The helminth Schistosoma haematobium can induce urothelial dysplasia and inflammation upon intravesical administration in mice [42] and has been linked to bladder cancer [43]. In another example, Propionibacterium acnes, found in prostate cancer and benign hyperplasia samples, when co-cultured with prostate epithelial cells results into production of pro-inflammatory cytokines, prostaglandins, and activated matrix metalloproteinases (MMPs), while long-term infection leads to anchorage-independent cancer cell growth [44]. Inflammation induced by chronic infections may be able to trigger mutations, epigenetic changes and protein modifications that may lead to oncogene activation and tumour suppressor inhibition [3]. Apart from the typical infectious agents, altered intestinal microbiota may also promote carcinogenesis, DNA damage and cell proliferation via chronic inflammatory processes [45]. Secretion of pathogen-induced cytokines may also have a dual role depending on the settings; for example, TNFα can mediate tumour haemorrhagic necrosis and regression [46, 47], while, on the other hand, it can promote carcinogenesis if present in a chronic fashion [48].

In addition to giving rise to the inflammation-mediated detrimental effects, pathogens may also promote tumourigenesis by inhibiting host anti-cancer immunity, for instance, by stimulating production of immunosuppressive cytokines (e.g. IL-10), causing T cell apoptosis, promoting T cell subtypes with attenuated anti-tumour activity (e.g. Th2) or triggering
recruitment of myeloid suppressor cells and Tregs [49-52]. Another potential effect on anti-tumour immunity triggered by chronic infections, also observed in cancer, is the dysfunction and subsequent elimination of antigen-specific T cells, a phenomenon called T cell exhaustion [53].

Infection in cancer prevention

Several observations, reported as early as the 1700s, support the link between infection-mediated inflammation and cancer prevention or regression (Table 1); most notably, efforts by William Coley in early 19th century to vaccinate his cancer patients with an attenuated bacterial mixture (*Streptococcus pyogenes* and *Serratia marcescens*) that accomplished significant cure and favourable progression rates [47, 54]. There is also evidence of the anti-tumour effect of certain microbial products (e.g. LPS) and attenuated pathogen forms [e.g. BCG (Bacillus Calmette-Guérin) vaccine] [13, 28, 55]; for instance, BCG, vaccinia or yellow fever virus vaccinations have been linked to melanoma protection [56, 57]. In addition, infectious agents have also been inversely associated with cancer (Table 1), as in the case of *Trypanosoma cruzi*, which can result in lower incidence of experimentally-induced rodent colon cancer [58]. These observations support the protective action of infections, as proposed by the hygiene hypothesis [11-14]. In the subsequent sections we will expand on this discussion by suggesting potential mechanisms that are often underestimated but may likely explain the favourable association of infection to carcinogenesis (Figure 1).

**Suppression of inflammation.** Several microbial products (e.g. lysophosphatidylserine) can have anti-inflammatory effects [34, 59]; for instance, they can suppress Toll-like receptor (TLR) signaling, inflammatory cytokine and nitric oxide production, as well as inhibit innate immune cell activation, stimulate production of immunosuppressive cytokines and recruitment of Tregs.
In this regard, Th1 or Th2 responses to some helminth infections rarely result in severe pathology [36] and can, in fact, downregulate allergic or autoimmune pathology (e.g. [60]). However, immunosuppressive cytokines specifically, have pleiotropic effects on tumourigenesis either by inhibiting inflammation-associated tumourigenesis, or by restricting anti-tumour immunity; for example, IL-10 is known to either inhibit or promote tumour growth, as well as facilitate tumour rejection in immune mice [61].

Promotion of anti-tumour immunity. Microorganisms may provide specific triggers (e.g. low level endotoxin, commonly produced by many pathogens) that increase antigenicity to nascent tumour cells, or keep the immune cells in an “alerted” immunosurveillance state [13]. This phenomenon resembles the infection-mediated stimulation of autoimmunity as a result of molecular mimicry, epitope spreading, exposure of cryptic antigens or bystander activation [62]. Epitope spreading has been observed in few cancer vaccine studies, i.e. following injection of dendritic cells in melanoma patients [63]. An infection can also lead to tumour cell destruction, subsequent release of tumour antigens, and activation of antigen-presenting cells. This could potentially trigger T cell responses with anti-tumour activities, like the ones that may be responsible for the protective action of BCG [64]. Moreover, potential increases in tumour vascular permeability may also facilitate the local recruitment of anti-cancer T cells [47]. Heat shock proteins, expressed by stressed cells and found upregulated in virus-infected and several cancer cells can also be immunogenic, thus influencing anti-tumour responses [65].

Presentation of cross-reactive antigens. Several pathogens contain antigens, mainly glycoproteins, that cross-react with tumour-associated antigens. As an example of such glycoprotein cross-reactivity, the Thomsen-Friedenreich T and Tn parasitic antigens can be detected in >80% of cancer patients and have been under experimental and clinical investigations
as markers and therapeutic targets for cancer [66, 67]. Furthermore, sera from patients suffering from parasitic infections (e.g. Echinococcus) are commonly found to cross-react (contain similar immunogenic epitopes) with sera from cancer patients [68]. Interestingly, it has been observed that such sera are more frequent in patients with less extensive malignancy. Antibodies against these shared parasite/tumour-associated antigens can potentially target tumour cells for destruction, or promote antigen presentation to T cells and induce anti-tumour responses; this antibody-mediated immune enhancement has been observed for non-tumour antigens in experimental models [69].

**Induction of pre-immunity.** The “concomitant immunity hypothesis” was originally suggested to explain resistance to secondary tumours or infections, particularly in animal models [10, 70]. As an ongoing persistent infection can protect the host from the same infection, similarly, in animal models, immunity to the original tumour can prevent growth of a comparable mass [10, 71]. Concomitant immunity was considered the result of either immunogenic, e.g. due to the presence of common antigenic epitopes, or non-immunogenic factors, such as in the presence of putative anti-mitotic components [10]. The concomitant effect may be abrogated once the original tumour is removed. It has also been observed that anti-cancer immunity can also be present after removal of the original malignant mass, a phenomenon termed sinecomitant immunity [10, 71], that can potentially be attributed to the parallel removal of tumour-induced immunosuppression.

**Formulation of the tumour microenvironment.** In principle, any agent that modulates antigen expression and cell populations in the tumour microenvironment can determine the quality and level of anti-cancer immunity. For instance, the previously observed effect of Coley’s toxin on cancer regression may be the result of TNFα, affecting local vascular permeability and
enhancing leukocyte recruitment [47, 54]. Microorganisms, such as helminthes and commensals, may also contribute to a cancer-inhibitory microenvironment by affecting Th1/Th2 responses and Tregs recruitment [36, 72]. Infection-mediated anti-tumour immunity can, however, be restricted by the immunosuppressive microenvironment that is often associated with developed tumours and characterized by the presence of myeloid-derived suppressor cells (MDSC) and Tregs [73]. Tumour-associated macrophages can also promote angiogenesis, tumour cell invasion, metastasis, and T cell inhibition. Angiogenesis itself has been related to immune suppression; for example, VEGF may lead to decreased antigen-presentation to T cells, due to inhibition of dendritic cells maturation [74]. The components of the tumour microenvironment can also direct the Th1/Th2 balance and promote Th2 responses with decreased anti-tumour properties [73]. The role of microbial infections in forming the local versus systemic or “secondary” (non-infected site) pro- or anti-carcinogenic immune milieu in competition with the immunosuppressive tumour microenvironment remains to be discovered.

**Production of low-level “danger” signals.** A phenomenon termed “hormesis” has been coined to describe a biphasic dose-dependent response to an agent characterized by a low-dose beneficial effect and a high-dose inhibitory or toxic effect [9]. It can be speculated that microbes, and specifically relatively benign microorganisms and commensals, embody this pleiotropic response stimulating DNA and tissue repair processes at low infectious agent loads, while resulting in extensive inflammatory and genomic changes that can subsequently foster pro-carcinogenic processes at higher pathogen loads. Interestingly, it has been postulated that the hygiene hypothesis describes this beneficial low-level exposure phenomenon [75]. As the hormeric effect would be highly dependent on spatial and temporal factors, in the case of carcinogenesis, both tumour stage and location at the time of infection may be of paramount
importance; the beneficial effects of pathogen-triggered stress repair processes at tumour
initiation may, therefore, be replaced by detrimental effects in later stages, when repair may be
accompanied by a more immunosuppressive microenvironment.

**Removal of carcinogens.** The health benefits of bacterially-enriched food (probiotics) and
certain ingredients that can stimulate growth of indigenous commensal bacteria (prebiotics) have
been widely discussed in several settings, including inflammation [76]. Although the evidence
for the ability of probiotics to reduce risk of colorectal cancer is still controversial, probiotics,
particularly containing bifidobacteria and lactobacilli, and prebiotics have been suggested to
reduce the production of carcinogens by other gastrointestinal bacteria like clostridia and
bacteroides [77].

**Inhibition of angiogenesis.** It has been suggested that infection can prevent angiogenesis, an
effect which may subsequently lead to restriction of tumour growth. For example, despite its
potential role in induction of tumour-promoting myeloid suppressor cells [78, 79], *Toxoplasma
gondii* infection is also known to suppress vascularization in a mouse melanoma model, an effect
that may be attributed in part to secretion of anti-angiogenic cytokines [80].

**Cancer immunotherapy and pathogen-based therapeutics**

The concept of using anti-inflammatory agents to regulate not only immune processes but
also the tumour load is not new, with the most widely-discussed recent example being the
benefits of aspirin in carcinogenesis risk reduction [81]. The latest approach in immune-related
cancer therapy is to promote targeting of specific tumour antigens or stimulate the host immune
response to growing tumours using a number of different approaches [82, 83]. Several tumour
cell antigens, i.e. cancer-specific, differentiation, viral, and carbohydrate antigens, as well as
mutated and overexpressed proteins have been considered as potential vaccine candidates (e.g. [84]). In addition, antibody-based therapeutic agents with reduced immunogenicity have been designed to specifically recognize and destroy tumour cells directly or via their specific stromal or immunomodulatory effects [82]. T cells have also been investigated in cancer treatment, for example in leukaemia and melanoma patients [85], while Tregs from mice infected by selected pathogens (e.g. Helicobacter hepaticus) have exhibited anti-cancer activity [86].

In a more microbe-based approach, pathogens and their toxins have been tested as anti-tumour agents or as carriers for tumour-targeting therapies [87]. The concept behind this approach is to employ the infectious agent or its selected components as means to treat/prevent cancer. In this regard, the BCG vaccine, an attenuated form of Mycobacterium bovis, is now an FDA-approved agent for the first-line intravesical treatment of bladder cancer [55]. BCG in this context may have a role in stimulating the body's own anti-cancer immunity via enhancing Th1 cytokine production (e.g. IFN-γ, TNF-α) [88, 89]. Microbial components may also find applicability in preventing cancer, as in the case of the tumour-pathogen T/Tn antigen [90] and the bacterial endotoxin LPS [28]. More specifically for the T/Tn antigen, vaccination regimens based on this common microbe-tumour glycoprotein [66, 67] have been previously evaluated on breast cancer prevention [90]. Vaccination was accompanied by an increase of helper T lymphocytes and decrease of T suppressor/cytotoxic cell ratio, possibly leading to regulation of anti-tumour immune responses and subsequent prevention of breast cancer recurrence.

More recently, the helminth Trichuris suis has been under clinical and experimental investigation for its ability to alleviate diseases, such as inflammatory bowel disease (ulcerative colitis, Crohn’s disease), multiple sclerosis and allergy (e.g. [91, 92]. Its applicability to cancer
pathology, and more specifically to tumours of the gastrointestinal system, is a question open to future investigations.

**Conclusion and future perspectives**

Both protective and detrimental effects of microorganisms have been observed, many of them linked to various immune components. Overall, their effect may depend on the fine orchestration between induction and suppression of cancer-promoting or anti-tumourigenic immunity, as well as on the level of pathogen load and the timing between infection and cancer initiation. In this regard, cancer may be associated with the increased hygiene/decreased exposure to specific microorganisms, similar to what is known for autoimmune diseases and allergies.

That said, it should be noted that not all types of microorganisms are expected to have the same anti-carcinogenic effect; for example, viral infections seem thus far to be mainly pro-carcinogenic, in contrast to bacteria or parasitic worms that have a longer coevolution history with human species. Novel clinical studies are, therefore, needed to delineate the specific role of these relatively benign organisms into modulating the host immune response towards cancer prevention. The adjuvant and cross-reactive effects of parasites and commensals should be investigated in greater detail in order to identify potential novel therapeutic targets. Exploration of the immunogenic epitope availability orchestrated by these agents may also, in the future, assist in the development of personalized treatments and immunization strategies, that can be used to prevent, regress or slow-down cancer progression.
Acknowledgements

Due to space limitations, we apologize for not citing more reviews and original papers related to this topic.
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Table 1. Association between different pathologies and cancer, based on epidemiological and experimental studies.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Association with cancer</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infections</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Helminths and protozoa</td>
<td>Negative(^1)</td>
<td>[13, 58, 80, 93-95]</td>
</tr>
<tr>
<td></td>
<td>Positive(^2)</td>
<td>[43, 96, 97]</td>
</tr>
<tr>
<td>Viruses</td>
<td>Negative(^1)</td>
<td>[57]</td>
</tr>
<tr>
<td></td>
<td>Positive(^2)</td>
<td>[2, 5, 13, 98]</td>
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<tr>
<td>Bacteria</td>
<td>Negative(^1)</td>
<td>[13, 26, 28, 47, 54-56]</td>
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<td></td>
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<td>[2, 5, 13, 27, 37, 98]</td>
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<tr>
<td>Allergy</td>
<td>Negative(^1)</td>
<td>[21-24]</td>
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<td></td>
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<td>[21, 24]</td>
</tr>
<tr>
<td>Autoimmune diseases</td>
<td>Positive(^2)</td>
<td>[19, 20]</td>
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</tbody>
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1. Negative: cancer prevention; cancer regression; decreased cancer risk.
2. Positive: cancer promotion; increased cancer risk
Figure Legend

Figure 1. Potential pathogen-mediated anti-tumour mechanisms. A microorganism may influence the fine balance between immunosuppression and immunity against a concurrent or subsequent tumour by modulating the availability and presentation of cross-reactive antigens and by shaping components of the tumour microenvironment. The levels of microbe-triggered stimuli are also decisive factors on the biphasic influence (pro- or anti-inflammatory) that a microorganism can have on immune functions. Several other mechanisms, such as removal of carcinogens and restriction of tumour vascularization may also facilitate the beneficial anti-tumour effects of microbes on their host.
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