Toward general prophylactic cancer vaccination

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It is well established that chronic infections can lead to cancer. Almost unknown is that, in contrast, acute brief viral and bacterial infections may have beneficial effects in cases of established neoplastic disease, while exposure to pathogenic products by infection, vaccination, and inhalation can cause prophylactic effects. In the following I will align evidence from case studies of spontaneous regression and from epidemiological studies with recent immunology to conclude that pathogenic substances belonging to the group of “pathogen-associated molecular patterns” can trigger the innate immune system to establish anti-neoplastic immune responses. A better understanding of the protective role of the innate immune system might leverage considerable prophylactic potential.

Keywords: cancer; DC maturation; fever; innate immune system; neoplasm; oncology; PAMP; prophylaxis; regression; Toll ligand; tumor

Introduction

The link between chronic infection and subsequent cancer has been established for certain pathogens, including papillomavirus and cervical cancer, Helicobacter pylori-induced gastritis and gastric cancer, inflammatory bowel disease and colorectal cancer, hepatitis B and C virus and hepatocellular cancer, and human herpesvirus and Kaposi sarcoma. Chronic inflammation was estimated to contribute to 15–20% of all malignancies. These links are part of medical linchpin, firmly entrenched into physicians’ minds, that pathogenic bacteria and viruses are evil, no matter what, and must be engaged in battle. Hence, any attempt to claim benefit for certain infections in some cancer patients might appear iconoclastic. Yet, cumulative evidence from different fields – case studies of spontaneous regression, epidemiology, and immunology – reveals that pathogens may be a two-edged sword with respect to cancer, because in contrast to chronic infection, acute, fully cleared infections may have beneficial effects.

In 1950 Shear posed the question: “Are pathogenic and non-pathogenic microorganisms one of nature’s controls of microscopic foci of malignant tissue, and, in making progress in the control of infectious diseases, are we not removing one of nature’s controls of cancer?” This is a valid and, given the lag of more than 50 years between hypothesis and confirmative evidence, a lucid statement, and the implications for cancer prophylaxis are profound.

Therapeutic effects of pathogens: spontaneous regression and post-operative infection

Shear as well as Diamond and Luby, in the early 1950s, had observed remissions in about 10% of children with untreated leukemia and noted that about three out of four such remissions occurred after an acute infection. Shear was not the first to report a connection in time between a hefty feverish infection and spontaneous regression; similar observations date back more than 150 years. A comprehensive literature investigation on more than 1,000 cases of spontaneous regression and remission in 2001 confirmed this link in at least 25–80% of cases, plus a putative number of spontaneous regressions with infections not reported. Until recently it was estimated that spontaneous regressions in adults are rare, but in a carefully designed study on mammography screening for female breast cancer (about 218,000 screened patients in Norway 1992–2001), spontaneous regression was estimated to happen in 22% of all cases of invasive breast cancer. This frequency is of similar magnitude as the number reported by Shear in 1951 for leukemia in children; however, the breast cancer study did not reveal whether, and if any, how many of these spontaneous regressions were connected with a feverish infection. Infections can have therapeutic effects not only before, but also after surgery, leading to longer survival upon post-operative infections.

Could a correlation in time between infection and spontaneous regression be in fact a causal connection? This assumption is supported by two additional lines of evidence:
old experiments with bacterial extracts in cancer patients and epidemiological findings (Table 1). Since the overlap between these three independent observations is a feverish viral or bacterial infection, there should be an immunological link between infection and immune rejection of cancer cells. The molecular link, most likely, are “pathogen-associated molecular pattern” (PAMP) molecules, also called Toll ligands, which are found in all pathogens but not in human tissues, and which are the most powerful activators of the innate arm of the immune system.

**Therapeutic effects of pathogenic molecules: therapeutic vaccination using bacterial extracts**

Probably inspired by the first report of a deliberate infection of a cancer patient in 1868(5) and starting in the 1890s continuing over a period of four decades, Coley and contemporaries inoculated hundreds of cancer patients with bacterial extracts generated from *Streptococcus pyogenes*. The dosage was adjusted to achieve a body temperature of more than 39°C, and injections were repeated once or twice a week, with treatment lasting up to several months. Without doubt, they achieved spectacular cures even in inoperable, late-stage cancer patients, but failed also in many other cases (reviewed in ref. (9,16–18)). Overall, Coley’s method performed surprisingly well. To compare the success rate of Coley’s cases with contemporary medicine, Richardson et al. tried to match 128 Coley cases with 1,675 controls from the Surveillance Epidemiology End Result (SEER) population-based cancer registry. Groups were matched on age, sex, ethnicity, stage, and radiation treatment status. Median survival was 8.9 (Coley) and 7.0 years (SEER), respectively.

However, the case-to-case unpredictability, strong opposition from within the medical community together with hypes and hopes originating from the invention of X-ray treatment at...
the beginning of the 20th century led to rebuff of the method after Coley’s death in 1936.

Besides *S. pyogenes*, several bacterial, fungal, viral, and pathogens have been tested until the 1960s as cancer therapy in humans (and many more in rodents), including *Serratia marcescens*, *Escherichia coli* (“pyrifer”), *Trypanozoma cruzi*, *Aspergillus niger* and Egypt virus (see²⁰ for review). None of these studies were performed according to present-day standards, but without doubt several remissions and even cures with survival rates exceeding 5 years were achieved,²¹ besides many failures. No consensus emerged on a favorable pathogen or therapy regimen. Results were, overall, unpredictable and success remained unexplained or was explained by a hypothetical pathogenic product called “cancer antibiotic”. In contrast to Coley, the majority of clinicians did not try to induce fever; however, for some of the lip and breast cancer cures achieved by Kluyeva using *Trypanosoma* extracts, temperature elevations were reported after i.m. injections.²¹

Some isolated attempts were made to revitalize Coley’s ideas in the 1960s, but the main lessons to be learned from Coley, namely to treat patients over many weeks and attempt to induce high fever, were not respected in these trials. Furthermore, most of these patients were immune-compromised by prior treatment with chemotherapy or radiation. Results were variable, with few cures, some responses, many failures, but were overall less encouraging than Coley’s results.⁹⁹ Tests using pathogens different from *Streptococcus* and perhaps uninspired by Coley’s treatment regimen followed. In the 1970s, BCG, a tuberculosis vaccine based on *Mycobacterium bovis*, was tested in cancer patients. *M. bovis*, unlike *S. pyogenes*, does not provide superantigens, and its immune-stimulating capability may be compromised by prior tuberculosis vaccination during childhood. Again results were variable²²–²⁴ and the method was discontinued, with the notable exception of intravesical BCG treatment for bladder cancer.²⁵⁵

A genetically modified strain of *Salmonella typhimurium* known to target tumors and inhibit tumor growth in mice²⁶ was tested in 24 melanoma patients in 2002.²⁷ No anti-tumor effects were seen, but in striking contrast to Coley’s regimen, fever was regarded as a dose-limiting toxicity, the LPS gene was genetically modified likely leading to reduced activation of the innate immune system, and the therapeutic goal was set very ambitious by defining clinical response as 50% decrease in lesions lasting minimally 1 month after a single bolus infusion.

and the likelihood to develop cancer later in life can be found (see Table 1). Publications linking infections and reduced subsequent cancer risk are distributed over many years and were, to this end not interpreted to represent solid evidence of a prophylactic effect. Again, no molecular explanation of any association was available. Yet, it seems problematic to reject these findings entirely. As an ensemble these epidemiological studies support the assumption that pathogens or products of pathogens can lower the risk of developing cancer later in life.

Exemplified by the work of Koelmel et al., it appears that the protective effect correlates with both number and severity of infections, the latter measured as fever height and duration,²⁸ and that the protective effect diminishes when the infection(s) occurred long ago during childhood, *i.e.*, when the development of cancer was preceded by a long non-febrile period.²⁹ The protective effect of infections might result in cleaning from pre-malignant tissue – a hypothesis so far.

Vaccines are meant to resemble an acute infection without the danger. There is some preliminary indication that vaccines might, similar to acute infections, provide some protection from cancer. Koelmel et al. report a reduced risk to develop melanoma after vaccinations with BCG and/or vaccinia virus, and among melanoma patients the survival time after resection of the primary tumor was significantly longer in those who were vaccinated.²⁰⁰ Recently, a similar, though statistically insignificant, protective effect was found for yellow fever vaccine inversely correlating with cancer risk.²¹ Yellow fever vaccine leads to febrile reactions in about 10–25% of vaccinations. However, the data basis was small, and protective effects were non-linear with time (stronger protection for vaccination more than 10 years ago compared to shorter intervals), a finding not easy to explain within the hypothetical frame presented here.

In line with this are findings about alleged protective effects of inhaled bacterial substances. In a case-control study²³ the odds ratio (OR) for lung cancer was significantly lower for dairy farmers in New Zealand, but not for crop/orchard farmers. Similar data were collected in Iceland,²³⁵ Sweden,²³⁴ and New York State.²³⁶ Mastrangelo et al. confirmed and extended this analysis showing that odds for lung cancer among farmers in Italy decreased with exposure, measured as working years and number of dairy cattle per farm.²³⁷ Levels of bacterial endotoxin in air can be seven times higher during livestock farming compared to field crop and fruit farming,²³⁸ and inhalation of dairy farm dust can lead to febrile reactions.

### Prophylactic effects of pathogens: infection, vaccination epidemiology, and endotoxin inhalation

Several epidemiological findings, which indicate an inverse correlation between a personal history of feverish infections and the odds ratio (OR) for lung cancer was significantly lower for dairy farmers in New Zealand, but not for crop/orchard farmers. Similar data were collected in Iceland,²³⁵ Sweden,²³⁴ and New York State.²³⁶ Mastrangelo et al. confirmed and extended this analysis showing that odds for lung cancer among farmers in Italy decreased with exposure, measured as working years and number of dairy cattle per farm.²³⁷ Levels of bacterial endotoxin in air can be seven times higher during livestock farming compared to field crop and fruit farming,²³⁸ and inhalation of dairy farm dust can lead to febrile reactions.

### Aligning case studies on spontaneous regression, epidemiology, and immunology

Today, we can offer an immunological hypothesis wiring all these findings together. Malignantly transformed cells carry...
hundreds of mutations; thus cancers are, in many cases, not invisible to the immune system, as indicated by tumor-infiltrating lymphocytes (TIL) around and inside a tumor. At least a portion of TIL consist of cytotoxic lymphocytes (CTL), indicating an active and specific immune response, with higher numbers of TIL directly translating into longer survival. This is a profound observation, since it proves that, in principle, the human immune system can reject cancer cells even at advanced stages, at least partially, in some patients, for a while. Nevertheless it is obvious that the normal immune response is usually too weak to eliminate an established neoplasm completely. But with help from viral or bacterial infection it seems, in principle, to be capable to stop or reverse growth of an established neoplasm (spontaneous regressions and post-operative infections) and to decrease the amount of pre-cancerous cells (epidemiological findings, Table 1). These effects are probably mediated by PAMP.

Pathogen-associated molecular patterns

PAMP denotes a collection of diverse molecules produced by pathogens, but not human tissues, including lipopolysaccharide (LPS) from Gram-negative bacteria, zymosan- and mannan from infectious fungi, bacterial flagellin, viral double-stranded RNA, CpG-DNA typical for bacterial genes, Trypanosoma glycoinositolphospholipids, and many others. PAMP bind to pattern recognition receptors (PRR) including Toll-like receptors (TLR), NOD-like receptors, C-type lectins, mannose receptors, retinoic acid-inducible gene 1 protein, and melanoma differentiation associated gene-5. From these PRR families, the TLR are, so far, the best investigated receptor family. TLR are prominently expressed on immune cells of the innate arm including macrophages and dendritic cells (DC), but also on some epidermal cells including fibroblasts. Binding of PAMP to professional antigen-presenting cells (APC) such as DC results, in this order, in phagocytosis and processing of microbial antigens to peptides, loading on MHC-I and MHC-II receptor molecules and display on the cell surface, upregulation of CCR7, migration through lymphatics to lymph nodes, expression of co-stimulatory molecules such as CD80 and CD86, scanning of the T-cell repertoire and activation of naive CD4+ and CD8+ T-cells.

It is important to underline that T-cells need three signals to engage in full effector function. The first signal is the antigen-specific signal induced by binding of the T-cell receptor to the MHC-peptide complex on the surface of DC. The second signal are co-stimulatory molecules expressed on DC triggering CD28. The third signal consists of inflammation-induced cytokines such as TNF-α or type-I interferons or cytokines delivered by APC including IL-6, IL-12, and TGF-β.

Without co-stimulation, antigen-specific T-cells are either not activated and even become unresponsive or, when activation occurs, lack effector function, which is exactly what can be observed in many cancers. While it is possible to induce upregulation of co-stimulatory molecules and MHC by inflammatory cytokines alone, that is without involvement of PAMP this is not sufficient for induction of T-cell effector differentiation. PAMP are critical in the generation of adaptive immunity.

Successful rejection of cancer cells requires appropriate involvement of the innate immune system

There is a long and ongoing discussion of why the immune system is well able to detect cancerous tissue, indicated for instance by frequent, sometimes massive tumor infiltration by lymphocytes including CTL (cognate immune response), but usually not capable to reject (effector immune response) a clinically evident malignancy completely. Tumor antigen-specific T-cells have a quiescent non-cytotoxic phenotype. This failure of eradication, despite effective recognition, has been mainly attributed to the numerous escape mechanisms which tumors can develop, including the suppression of tumor antigen cell surface display, attraction of regulatory T-cells to downregulate local immune responses, suppression of cell surface expression of stress proteins like MICA needed to fully activate natural killer cells and development of resistance to FasL-mediated killing. But spontaneous regressions and remissions, which sometimes lead to cure, show that these escape mechanisms can, in principle, be overcome. Accordingly, there must exist a mechanism to handle even clinically evident tumors. PAMP might be the missing link, since DC are best stimulated by PAMP. Hence, PAMP recruit the innate arm of the immune system, which is normally not involved in immune suppression of malignant growth.

TLR-triggered apoptosis, originally reported in plants, where this mechanism is used to confine pathogens, exists in cancer cells. Whether this mechanism plays a relevant role in cancer cell death upon infection – cancer cells often exploit shunning apoptotic pathways as a main escape mechanism remains to be investigated.

DC in cancer patients usually remain immature. This may be due to slow antigen level increase, low final antigen level, lack of PAMP, or a combination of those. For maturation, DC need, besides PAMP relatively high amounts of stable antigen to become fully activated. Since chemotherapy and radiotherapy lead to increased tumor cell death, it has been suggested to combine the administration of exogenous TLR ligands with chemo- or radiotherapy; however, the immune-compromising effects of chemo- and radiotherapy on DC maturation were not addressed.
The role of fever or externally applied heat

Immune-compromising effects of chemo- and radiotherapy might be reduced and beneficial PAMP effects enhanced by fever or externally applied heat (hyperthermia), as indicated by the following observations. Tumor cells are usually less heat-resistant than normal cells, therefore die to a larger extent under fever\(^\text{(55)}\) and presumably supply a sudden rise in tumor antigens, lifting antigen level closer to that needed for DC activation. A higher load of immunogenic HSP-peptide complexes is displayed on some cancer cell lines after heat treatment.\(^\text{(56,57)}\) These complexes can activate natural killer cells against human lung carcinoma cells, opening a second avenue of innate response independent of MHC-restricted immunogenicity.\(^\text{(58)}\) Basu and Srivastava showed in 2003 that heat (39.5 or 41 °C for 6–12 hours) induces stronger cell surface expression of maturation markers and MHC-II on DC, better antigen presentation and as a result stronger T-cell stimulation.\(^\text{(59)}\) Stronger T-cell stimulation was as well observed when melanoma cells, rather than DC, were heated to 42 °C,\(^\text{(60)}\) so one might expect synergistic effects upon heating both cancer cells and DC.

There are also hints that TLR-activated DC inhibit suppressive effects of regulatory T-cells,\(^\text{(61)}\) thus potentially countering one of the main defense mechanisms malignant tissue can engage.

Can pathogenic substances lead to stronger reactivity against tumor antigens?

The crucial question is: can PAMP lead to activation and clonal expansion of tumor-specific effector T-cells? Common understanding is that the only T-cell clones to be activated should be pathogen-specific.

One explanation might be a hypothetical cross-activation of tumor-specific T-cells (see Fig. 1). When a hefty feverish infection occurs in a cancer patient, the innate arm becomes engaged. Since APC are not known to be selective with respect to antigen, they will likely collect all antigens in their vicinity, namely both pathogen- and tumor-specific antigens, leading to activation of both pathogen- and tumor-specific T-cells. While this model has not been proven directly, there is suggestive supportive evidence.

In an attempt to break tumor tolerance, Pardoll and coworkers injected DC into mice with HA antigen-expressing lymphoma using three different experimental settings. In the first setting, DC were pulsed with HA antigen; in the second setting, DC were infected with HA-expressing lentivirus. Tolerance could be broken in the second setting, not the first, indicating that PAMP produced by the virus were required to break tolerance. This conclusion was supported by the results

**Figure 1.** A: Binding of both antigen and TLR ligands to MHC and TLR, respectively, are required to induce generation of co-stimulatory signals (expression of B7 and release of cytokines) by DC and subsequent activation of T-cells. B: In cancer patients, tumor antigens can often be found, but TLR ligands are usually missing. C: Upon infection of a cancer patient, TLR ligands are supplied and might aid in full activation of tumor specific T-cell clones.
from the third setting, a variant of setting one: if DC pulsed with tumor antigen were co-injected with LPS, a PAMP molecule known to generate vigorous inflammatory response, tolerance could likewise be broken. Many other DC vaccination attempts, similar to the first setting, showed that tumor antigen alone or DC loaded with tumor antigen are not enough to engage a powerful immune defense against tumor cells, while Pardolls results indicate that DC vaccination strategies involving antigen plus PAMP can be effectual. In a pancreatic mouse tumor, S. pyogenes applied by single intratumoral injection led to complete remission when live bacteria were used, and growth delay when lysed bacteria were administered. Together, these findings align with Matzingers blunt prediction "to eradicate a tumor, we should infect it."(66)

Safety of PAMP

Pathogen antigens and cancer antigens were tested in phases I–III clinical trials together with Toll ligands, including CpG-ODN (nucleotide PAMP) combined with Melan-A peptide or influenza antigens or inactivated HIV or anthrax vaccine; monophosphoryl lipid (MPL PAMP) combined with cancer-associated MUC1 protein or human papillomavirus particles or human hepatitis B virus antigen; imidazoquinoline (synthetic PAMP) coupled with HIV-Gag protein; and flagellin (protein PAMP) combined with influenza hemagglutinin. In these cases, Toll ligands were meant to act as adjuvant, not main actors. In a few cases, single Toll ligands were tested in clinical trials. These were patented CpG derivatives (nucleotide PAMP) including Aldara (basal cell carcinoma), Imoxine (renal cell carcinoma), and resiquimod (Herpes simplex infection, chronic hepatitis C). Overall, no severe side reactions were reported. Therapeutic benefit was limited, but therapy regimens were not optimized according to the lessons to be learned from historic therapeutic vaccinations, in particular to stimulate the innate system multiple times with combinations of PAMP (for discussion see ref.69).

Combinations of PAMP have been tested in rodents with synergistic effects on cytokine patterns: poly(I:C) (TLR3) or LPS (TLR4) was synergistic with R-848 (TLR8) and caused synergistic effects on cytokine patterns: poly(I:C) (TLR3) or R-848 (TLR8) combined with influenza hemagglutinin, imidazoquinoline (synthetic PAMP) coupled with HIV-Gag protein; and flagellin (protein PAMP) combined with influenza hemagglutinin. In these cases, Toll ligands were meant to act as adjuvant, not main actors. In a few cases, single Toll ligands were tested in clinical trials. These were patented CpG derivatives (nucleotide PAMP) including Aldara (basal cell carcinoma), Imoxine (renal cell carcinoma), and resiquimod (Herpes simplex infection, chronic hepatitis C). Overall, no severe side reactions were reported. Therapeutic benefit was limited, but therapy regimens were not optimized according to the lessons to be learned from historic therapeutic vaccinations, in particular to stimulate the innate system multiple times with combinations of PAMP (for discussion see ref.69).

Multiple PAMP act synergistically

As indicated above, the involvement of multiple Toll receptors can induce cytokines synergistically. Some Toll receptors signal as heterodimers upon binding of different PAMP ligands, for instance TLR1/TLR2 and TLR5, which are the natural ligands of flagellin, phospholipomannan, and TLR2/TLR6, which are the natural ligands of TLR2/TLR5, respectively. These ligands are thought to function as heterodimers upon binding of different PAMP ligands. The involvement of multiple Toll receptors in the immune response to pathogens is well documented. The involvement of multiple Toll receptors in the immune response to pathogens is well documented. The involvement of multiple Toll receptors in the immune response to pathogens is well documented. The involvement of multiple Toll receptors in the immune response to pathogens is well documented. The involvement of multiple Toll receptors in the immune response to pathogens is well documented. The involvement of multiple Toll receptors in the immune response to pathogens is well documented. The involvement of multiple Toll receptors in the immune response to pathogens is well documented. The involvement of multiple Toll receptors in the immune response to pathogens is well documented. The involvement of multiple Toll receptors in the immune response to pathogens is well documented. The involvement of multiple Toll receptors in the immune response to pathogens is well documented.
of three or more infections with high fever above 38.5 °C (mean OR 0.16 for pulmonary tuberculosis, 0.23 for sepsis, 0.45 for pneumonia, 0.54 for Staphylococcus aureus infections, 0.65 for influenza and related infections, higher OR for infections without or lower fever).(28)

A more recent study showed a strong protective effect of infection after cancer surgery: the 10 years survival for osteosarcoma patients who had an infection within a time frame of 1 year after surgery (n = 41) was 84.5% compared to 62.3% in the non-infected group (n = 371), i.e., a risk reduction of about 27%.(14) This study is in line with an earlier one showing that in patients developing empyema after lung cancer surgery, 5 years survival was more than doubled compared to control (50%, n = 18 vs. 22%, n = 411). These findings indicate that protection impacts not only on pre-cancorous cells but also on residual malignant foci after treatment.

Inhalation of cattle dust by Italian farmers, presumably loaded with high levels of microbial substances and leading to common febrile reactions, leads to a standardized mortality ratio from malignant tumors of 0.67, i.e., a significant decrease in 33%。(37)

Taken together, putative prophylactic effects of pathogenic substances, with a particular focus on PAMP, may be weighty.

Conclusions

Since a lower incidence of cancer translates directly into lower mortality, we should channel appropriate intellectual and financial efforts toward prophylaxis as well as toward therapy. Cancer incidence and mortality have not changed dramatically over the last 50 years, despite immense expense for research and treatment. The frequency of all forms of cancer in the US as cause of death (mortality) decreased by only 5% between 1950 and 2004 (all races, both sexes, all primary neoplasms). To mimic a proliferative infection, multiple PAMP shots or inhalations in brief succession might be needed. To exploit immunostimulatory effects of fever, body temperature elevation should be aimed at as a marker rather than disapproved as adverse side reaction. In case of positive outcome, routine PAMP vaccinations in humans aimed at stimulating both parts of the immune system against pre-cancorous cells could be envisaged. The putative requirement of fever will interfere stronger with modern life style than ordinary vaccinations and ideally require individual dosing and 1 or 2 days rest at home. To pacify the employer, these could be scheduled during weekends or holidays.

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References

Problems and paradigms


