Toward general prophylactic cancer vaccination

*Uwe Hobohm**

University of Applied Sciences, Bioinformatics, Wiesenstrasse 14, D-35390 Giessen, Germany

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, *Heliobacter 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.[1] Chronic inflammation was estimated to contribute to 15–20% of all malignancies.[2] 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?''[3] 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.

***Correspondence to:** U. Hobohm, University of Applied Sciences, Bioinformatics, Wiesenstrasse 14, D-35390 Giessen, Germany.
E-mail: uwe.hobohm@tg.fh-giessen.de

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

Shear as well as Diamond and Luby,[4] 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.[5–8] 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.[9] 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.[10] 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.[11–14]

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:

Table 1. Prophylactic effects of acute infections (age-matched controls[28,79,80,81]).

Observation	Pathogen
Lower risk of cancer in syphilitic prostitutes[82]	*Treponema pallidum*
Low risk of cancer in tuberculosis patients[8]	*M. tuberculosis*
Lower risk of cancer in malaria patients[7,83]	*Plasmodium falciparum, malariae, vivax*
"According to the Cancer Centre in Sao Paulo (Brazil), among tens of thousands of cancer patients only two gave a positive Machado reaction (typical of ... patients recovered from trypanosome infection), whereas among the remaining population the number suffering from this infection varies from 10 to 20%" (anecdotal introductory remark in[21])	*Trypanosoma*
Lower cancer risk associated with a personal history of infections (232 cancer patients vs. 2444 controls)[84]	Diverse
Cancer risk decreased with history of common colds (OR 5.7) and history of fever (OR 15.1)[85]	Diverse
Within a group of 353 individuals without measles, 21 developed cancer *vs.* 1/230 controls with a positive history of measles ($p = 0.001$)[86]	Morbillivirus
Lower cancer incidence after Herpes infections[87]	Herpes simplex
A history of common colds or gastroenteric influenza was found to be associated with a decreased cancer risk (OR 0.18 and 0.23 *vs.* population and hospital controls, respectively)[88]	Common cold viruses
After limb-sparing surgery for canine osteosarcoma about half of the dogs developed nosocomial bacterial infections. Life expectancy was twice for infected dogs[89]	Nosocomial infections
Inverse correlation between number of infections and mortality from tumors in Italy in the period 1890–1960: each 2% reduction in number of infectious diseases was followed by a 2% increase in tumors about 10 years later[90]	Diverse
Statistically significant inverse association between a reported history of infections and glioma, meningioma (RR = 0.72, age- and gender-matched population control of 1,509 cases)[79]	Diverse
Inverse correlation between melanoma risk and number of recorded infections on one hand and between melanoma risk and fever height on the other hand, leading to a combined reduction of melanoma risk of about 40% for people with a history of three or more infections with high fever above 38.5 °C (age- and gender-matched population control)[28]	Diverse
Inverse relation between incidence of common infections acquired by exposure to other children in childcare facilities and childhood leukemia[80,81]	Diverse

Omitted is one publication reporting an increased risk of childhood rhabdomyosarcoma with feverish infections not reaching statistical significance[91] and a report in a non-peer-reviewed journal showing conflicting data for different pathogens.[92]

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,[15] 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.*[19] 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 (see[20] 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.[9] 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 variable[22–24] and the method was discontinued, with the notable exception of intravesical BCG treatment for bladder cancer.[25]

A genetically modified strain of *Salmonella typhimurium* known to target tumors and inhibit tumor growth in mice[26] 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.

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 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.[30] Recently, a similar, though statistically insignificant, protective effect was found for yellow fever vaccine inversely correlating with cancer risk.[31] 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 study[32] 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,[33] Sweden,[34] and New York State.[35,36] 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.[37] Levels of bacterial endotoxin in air can be seven times higher during livestock farming compared to field crop and fruit farming,[38] 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;[39] 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),[40] indicating an active and specific immune response, with higher numbers of TIL directly translating into longer survival.[41,42] 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 lipopolysaccaride (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.[43,44] 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.[45] 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.[46] 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[47] 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.[48] 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.[49] 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.[50] 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.[51] 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[52] – 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.[53] 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;[54] 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[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.[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.[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.[59] Stronger T-cell stimulation was as well observed when melanoma cells, rather than DC, were heated to 42 °C;[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,[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

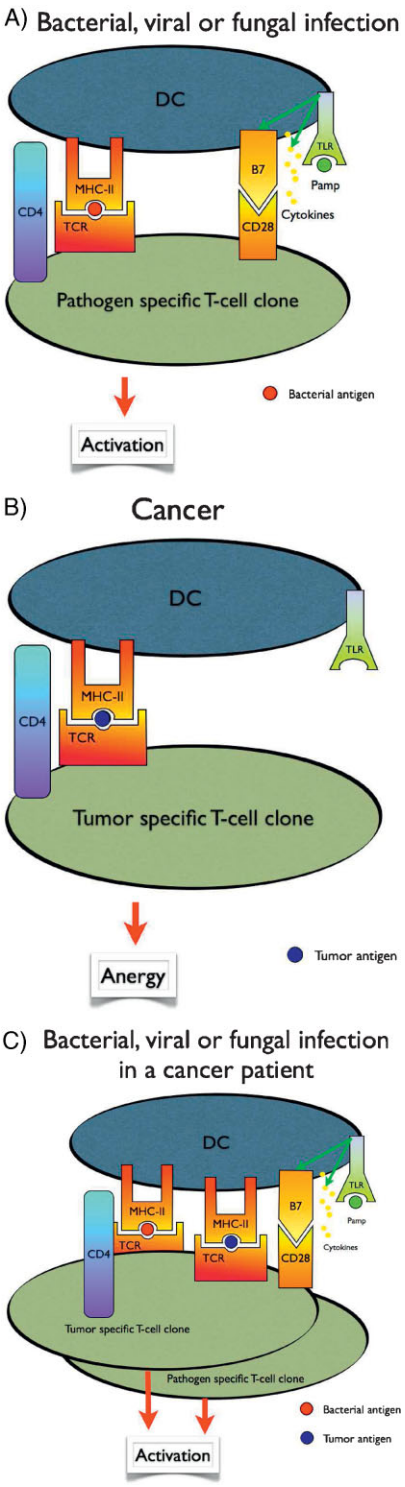

Figure 1. **A**: Binding of both antigen and TLR ligands to MHC and TLR, respectively, are required to induce generation of co-stimulatory signals (expression of B7 and release of cytokines) by DC and subsequent activation of T-cells. **B**: In cancer patients, tumor antigens can often be found, but 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.[62] 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,[63] 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,[64] and growth delay when lysed bacteria were administered.[65] 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; monophosphyryl 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.[67,68] 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).[67] 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 DCs to produce 50- to 100-fold more interleukins (IL-12p70, IL-23, IL-δ-4) than individual agonists. Only combined agonists were able to induce IL-1β processing; no severe side reactions were reported.[70] CpG PAMP were administered in doses of hundreds of milligrams on a weekly or monthly basis to Crohn's disease patients without adverse effects.[71]

Some caution is required for the potential application of LPS in combination with other substances. Sub-lethal doses of superantigen and LPS can synergistically induce toxic shock if co-administered;[72] CpG co-administered with sub-pathological doses of LPS and D-galactosamine gave rise to severe mortality in mice.[73] On the other hand, the maximum tolerated systemic dose of *S. abortus equi* LPS was 1 ng/kg in a phase-II study.[74] Dose-limiting toxicities were WHO grade-III fever, chills, and hypotension. With ibuprofen, the dose could be escalated to 5 ng/kg. Interestingly, an increased ratio of CD4/CD8 and TNF-β release could be upheld by repetitive LPS injections. In the Hufeland-Klinik in Bad Mergentheim (Germany), so-called fever therapy was applied to cancer patients for decades without any fatality (Dr. Wöppel, personal communication) using Vaccineurin, a commercial preparation similar to "Coley's toxins"; however, results were never evaluated statistically. Together, these results suggest that application of PAMP is quite safe.

Multiple PAMP act synergistically

As indicated above, the involvement of multiple Toll receptors can induce cytokines synergistically.[70] Some Toll receptors signal as heterodimers upon binding of different PAMP ligands, for instance TLR1/TLR2 (ligands: triacyl lipopetides, Pam2Cys, phospholipomannan) or TLR2/TLR6 (ligands: diacyl lipopeptides, lipoteichoic acid, zymosan).[43,44] Application of both TLR1 and TLR2 ligands in mice resulted in a synergistic antitumor effect involving tumor-specific CD8 T-cells.[75] Expression of TLR2 and TLR4 can be interdependent or follow a specific timeline depending on the pathogen.[76] Deletion or inhibition of a single TLR (1, 2, 4, 9, 2/6) does not impair cytokine secretion or upregulation of co-stimulatory molecules in mice DC infected by *S. pyogenes*,[77] strongly suggesting that several TLR are involved in sensing of streptococci. Engagement of multiple PAMP appears plausible to avoid false-positive alerts and to fine-tune immune responses against pathogens. Therefore, for cancer prophylaxis or treatment, best results can likely be expected with cocktails of PAMP or inactivated pathogens.

Implications for prophylaxis

Since the implications of this model for cancer therapy have been discussed elsewhere,[69] I will concentrate on prophylaxis here. If we allow for the hypothesis that products of pathogens might help the immune system against cancer, how strong might these prophylactic effects be? An age-adjusted comparison between 603 melanoma patients and 627 population controls revealed an inverse correlation between melanoma risk and the number of recorded infections on the one hand and between melanoma risk and the severity of infection, measured by height and duration of fever, on the other hand. There was a combined reduction of melanoma risk of about 40% in people with a personal history

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 precancerous 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 cancer sites combined[78]). The findings collected here indicate that acute feverish infections could reduce the risk of developing at least some forms of cancer by around 20–40%. Thus, even if a fraction of this protection would be realized by harnessing stimulators of the innate immune system like bacterial or viral or fungal PAMP, the reduction in cancer mortality could well exceed that due to all pharmaceutical efforts of the last 50 years (with the notable exception of some recent antibody therapies).

How such a protection could be put into practice is an open question. Suggestions to change attitude in life style like ''live less sterile and hygienic'' or ''allow for some unpleasant days in bed without antibiotics to cope with milder feverish infections'' might stir vigorous debate but still be serious, since we have to balance quick alleviation of discomfort with loss of long-term benefit. Careful examination in the mouse cancer model is required, with emphasis on prophylaxis, that is by testing PAMP against slowly growing spontaneous 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 precancerous 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.

Acknowledgments: There are no financial conflicts of interest that might be construed to influence the results or interpretation of this paper. I thank Prof. John Grange for critically reading the paper.

References

1. **Tan, T. T. and Coussens, L. M.,** Humoral immunity, inflammation and cancer. *Curr Opin Immunol* 2007. **19**: 209–216.
2. **Mantovani, A. and Pierotti, M. A.,** Cancer and inflammation: a complex relationship. *Cancer Lett* 2008. **267**: 180–181.
3. **Reinhard, E. H., Good, J. T.,** and **Martin, E.,** Chemotherapy of malignant neoplastic diseases – abstract of discussion, with statement by MJ Shear, Bethesda. *JAMA* 1950. **142**: 383.
4. **Diamond, L. K. and Luhby, L. A.,** Pattern of 'spontaneous' remissions in leukemia of the childhood, observed in 26 of 300 cases. *Am J Med* 1951. **10**: 238ff.
5. **Busch, W.,** Aus der Sitzung der medicinischen Section vom 13. November 1867. *Berliner Klinische Wochenschrift* 1868. **5**: 137.
6. **Rohdenburg, G.,** Fluctuations in the growth energy of malignant tumors in man, with especial reference to spontaneous recession. *J Cancer Res* 1918. **3**: 193–225.
7. **Braunstein, A.,** Krebs und malaria. *Z Krebsforsch* 1929. **29**: 330–333.
8. **Pearl, R.,** Cancer and tuberculosis. *Am J Hyg* 1929. **9**: 97–162.
9. **Hobohm, U.,** Fever and cancer in perspective. *Cancer Immunol Immunother* 2001. **50**: 391–396.
10. **Zahl, P. H., Maehlen, J. and Welch, H. G.,** The natural history of invasive breast cancers detected by screening mammography. *Arch Intern Med* 2008. **168**: 2311–2316.
11. **Ruckdeschel, J. C., Codish, S. D., Stranahan, A. and McKneally, M. F.,** Postoperative empyema improves survival in lung cancer. Documentation and analysis of a natural experiment. *N Engl J Med* 1972. **287**: 1013–1017.
12. **Rotoli, B., Formisano, S., Martinelli, V. and Nigro, M.,** Long-term survival in acute myelogenous leukemia complicated by chronic active hepatitis. *N Engl J Med* 1982. **307**: 1712–1713.
13. **Treon, S. P. and Broitman, S. A.,** Beneficial effects of post-transfusional hepatitis in acute myelogenous leukemia may be mediated by lipopolysaccharides, tumor necrosis factor alpha and interferon gamma. *Leukemia* 1992. **6**: 1036–1042.
14. **Jeys, L. M., Grimer, R. J., Carter, S. R., Tillman, R. M. and Abudu, A.,** Post-operative infection and increased survival in osteosarcoma patients: are they associated? *Ann Surg Oncol* 2007. **14**: 2887–2895.
15. **Christian, S. and Palmer, L.,** An apparent recovery from multiple sarcoma with involvement of both bone and soft parts treated by toxin of erysipales and bacillus prodigiosus. *Am J Surg* 1928. **43**: 188–197.

16. **Coley-Nauts, H. C., Fowler, G.,** and **Bogatko, F. H.,** A review of the influence of bacterial infection and of bacterial products (Coley's toxins) on malignant tumors in man. *Acta Med Scand* 1953. **145**: 5–102.
17. **Nauts, H. C. and McLaren, J. R.,** Coley toxins–the first century. *Adv Exp Med Biol* 1990. **267**: 483–500.
18. **Wiemann, B. and Starnes, C. O.,** Coley's toxins, tumor necrosis factor and cancer research: a historical perspective. *Pharmacol Ther* 1994. **64**: 529–564.
19. **Richardson, M. A., Ramirez, T., Russell, N. C. and Moye, L. A.,** Coley toxins immunotherapy: a retrospective review. *Altern Ther Health Med* 1999. **5**: 42–47.
20. **Reilly, H.,** Microbiology and cancer therapy: a review. *Cancer Res* 1953. **13**: 821–834.
21. **Klyuyeva, N. G. and Roskin, G. I.,** Biotherapy of malignant tumours. Oxford, Pergamon Press, 1963.
22. **Grant, R. M., Mackie, R., Cochran, A. J., Murray, E. L., Hoyle, D. and Ross C.,** Results of administering B.C.G. to patients with melanoma. *Lancet* 1974. **2**: 1096–1100.
23. **Mastrangelo, M. J., Sulit, H. L., Prehn, L. M., Bornstein, R. S., Yarbro, J. W. and Prehn, R. T.,** Intralesional BCG in the treatment of metastatic malignant melanoma. *Cancer* 1976. **37**: 684–692.
24. **Morton, D. L., Eilber, F. R., Holmes, E. C., Hunt, J. S., Ketcham, A. S.,** *et al.* BCG immunotherapy of malignant melanoma: summary of a seven-year experience. *Ann Surg* 1974. **180**: 635–643.
25. **Patard, J., Moudouni, S., Saint, F., Leclercq, N. R., Manunta, A.,** *et al.* Tumor progression and survival in patients with T1G3 bladder tumors: multicentric retrospective study comparing 94 patients treated during 17 years. *Urology* 2001. **58**: 551–556.
26. **Low, K. B., Ittensohn, M., Le, T., Platt, J., Sodi, S.,** *et al.* Lipid A mutant Salmonella with suppressed virulence and TNFalpha induction retain tumor-targeting in vivo. *Nat Biotechnol* 1999. **17**: 37–41.
27. **Toso, J. F., Gill, V. J., Hwu, P., Marincola, F. M., Restifo, N. P.,** *et al.* Phase I study of the intravenous administration of attenuated Salmonella typhimurium to patients with metastatic melanoma. *J Clin Oncol* 2002. **20**: 142–152.
28. **Koelmel, K. F., Pfahlberg, A., Mastrangelo, G., Niin, M., Botev, I.,** *et al.* Infections and melanoma risk: results of a multicenter EORTC case study. *Melanoma Res* 1999. **9**: 511–519.
29. **Kolmel, K. F., Gefeller, O.,** and **Haferkamp, B.,** Febrile infections and malignant melanoma: results of a case-control study. *Melanoma Res* 1992. **2**: 207–211.
30. **Koelmel, K. F., Grange, J. M., Krone, B., Mastrangelo, G., Rossi, C. R.,** *et al.* Prior immunisation of patients with malignant melanoma with vaccinia or BCG is associated with better survival. An European Organization for Research and Treatment of Cancer cohort study on 542 patients. *Eur J Cancer* 2005. **41**: 118–125.
31. **Mastrangelo, G., Krone, B., Fadda, E., Buja, A., Grange, J. M.,** *et al.* Does yellow fever 17D vaccine protect against melanoma? *Vaccine* 2009. **27**: 588–591.
32. **Reif, J., Pearce, N. and Fraser, J.,** Cancer risks in New Zealand farmers. *Int J Epidemiol* 1989. **18**: 768–774.
33. **Rafnsson, V. and Gunnarsdottir, H.,** Mortality among farmers in Iceland. *Int J Epidemiol* 1989. **18**: 146–151.
34. **Wiklund, K. and Steineck, G.,** Cancer in the respiratory organs of Swedish farmers. *Cancer* 1988. **61**: 1055–1058.
35. **Stark, A. D., Chang, H. G., Fitzgerald, E. F., Riccardi, K. and Stone, R. R.,** A retrospective cohort study of cancer incidence among New York State Farm Bureau members. *Arch Environ Health* 1990. **45**: 155–162.
36. **Wang, Y., Lewis-Michl, E. L., Hwang, S. A., Fitzgerald, E. F. and Stark, A. D.,** Cancer incidence among a cohort of female farm residents in New York State. *Arch Environ Health* 2002. **57**: 561–567.
37. **Mastrangelo, G., Grange, J. M., Fadda, E., Fedeli, U., Buja, A. and Lange, J. H.,** Lung cancer risk: effect of dairy farming and the consequence of removing that occupational exposure. *Am J Epidemiol* 2005. **161**: 1037–1046.
38. **Nieuwenhuijsen, M. J., Noderer, K. S., Schenker, M. B., Vallyathan, V. and Olenchock, S.,** Personal exposure to dust, endotoxin and crystalline silica in California agriculture. *Ann Occup Hyg* 1999. **43**: 35–42.
39. **Loeb, L. A., Loeb, K. R. and Anderson, J. P.,** Multiple mutations and cancer. *Proc Natl Acad Sci U S A* 2003. **100**: 776–781.
40. **Trojan, A., Urosevic, M., Dummer, R., Giger, R., Weder, W. and Stahel, R. A.,** Immune activation status of CD8+ T cells infiltrating non-small cell lung cancer. *Lung Cancer* 2004. **44**: 143–147.
41. **Black, M. S., Opler, S. and Speer, S.,** Structural representations of tumor-host relationships in gastric carcinoma. *Surg Gynec Obstet* 1956. **102**: 599–603.
42. **Dunn, G. P., Bruce, A. T., Ikeda, H., Old, L. J. and Schreiber, R. D.,** Cancer immunoediting: from immunosurveillance to tumor escape. *Nat Immunol* 2002. **3**: 991–998.
43. **Akira, S., Uematsu, S. and Takeuchi, O.,** Pathogen recognition and innate immunity. *Cell* 2006. **124**: 783–801.
44. **Guy, B.,** The perfect mix: recent progress in adjuvant research. *Nat Rev Microbiol* 2007. **5**: 505–517.
45. **Blohm, U., Roth, E., Brommer, K., Dumrese, T., Rosenthal, F. M. and Pircher, H.,** Lack of effector cell function and altered tetramer binding of tumor-infiltrating lymphocytes. *J Immunol* 2002. **169**: 5522–5530.
46. **Gallucci, S., Lolkema, M. and Matzinger, P.,** Natural adjuvants: endogenous activators of dendritic cells. *Nat Med* 1999. **5**: 1249–1255.
47. **Alexander, P., Eccles, S. and Gauci, C.,** The significance of macrophages in human and experimental tumours. *Ann N Y Acad Sci* 1976. **276**: 124–133.
48. **Monsurro, V., Wang, E., Yamano, Y., Migueles, S. A., Panelli, M. C.,** *et al.* Quiescent phenotype of tumor-specific CD8+ T cells following immunization. *Blood* 2004. **104**: 1970–1978.
49. **Khong, H. T. and Restifo, N. P.,** Natural selection of tumor variants in the generation of ''tumor escape'' phenotypes. *Nat Immunol* 2002. **3**: 999–1005.
50. **Schuurhuis, D. H., Fu, N., Ossendorp, F. and Melief, C. J. M.,** Ins and outs of dendritic cells. *Int Arch Allergy Immunol* 2006. **140**: 53–72.
51. **Salaun, B., Romero, P. and Lebecque, S.,** Toll-like receptors' two-edged sword: when immunity meets apoptosis. *Eur J Immunol* 2007. **37**: 3311–3318.
52. **Letai, A. G.,** Diagnosing and exploiting cancer's addiction to blocks in apoptosis. *Nat Rev Cancer* 2008. **8**: 121–132.
53. **Kurts, C., Sutherland, R. M., Davey, G., Li, M., Lew, A. M.,** *et al.* CD8 T cell ignorance or tolerance to islet antigens depends on antigen dose. *Proc Natl Acad Sci U S A* 1999. **96**: 12703–12707.
54. **Melief, C. J.,** Cancer immunotherapy by dendritic cells. *Immunity* 2008. **29**: 372–383.
55. **Trieb, K., Sztankay, A., Amberger, A., Lechner, H. and Grubeck-Loebenstein, B.,** Hyperthermia inhibits proliferation and stimulates the expression of differentiation markers in cultured thyroid carcinoma cells. *Cancer Lett* 1994. **87**: 65–71.
56. **Udono, H. and Srivastava, P. K.,** Heat shock protein 70-associated peptides elicit specific cancer immunity. *J Exp Med* 1993. **178**: 1391–1396.
57. **Multhoff, G., Botzler, C., Wiesnet, M., Müller, E., Meier, T.,** *et al.* A stress-inducible 72-kDa heat-shock protein (HSP72) is expressed on the surface of human tumor cells, but not on normal cells. *Int J Cancer* 1995. **61**: 272–279.
58. **Botzler, C., Issels, R. and Multhoff, G.,** Heat-shock protein 72 cell-surface expression on human lung carcinoma cells is associated with an increased sensitivity to lysis mediated by adherent natural killer cells. *Cancer Immunol Immunother* 1996. **43**: 226–230.
59. **Basu, S. and Srivastava, P. K.,** Fever-like temperature induces maturation of dendritic cells through induction of hsp90. *Int Immunol* 2003. **15**: 1053–1061.
60. **Shi, H., Cao, T., Connolly, J. E., Monnet, L., Bennett, L.,** *et al.* Hyperthermia enhances CTL cross-priming. *J Immunol* 2006. **176**: 2134–2141.
61. **Kabelitz, D., Wesch, D. and Oberg, H. H.,** Regulation of regulatory T cells: role of dendritic cells and toll-like receptors. *Crit Rev Immunol* 2006. **26**: 291–306.
62. **Yang, Y., Huang, C. T., Huang, X. and Pardoll, D. M.,** Persistent Toll-like receptor signals are required for reversal of regulatory T cell-mediated CD8 tolerance. *Nat Immunol* 2004. **5**: 508–515.
63. **Srivastava, P. K.,** Therapeutic cancer vaccines. *Curr Opin Immunol* 2006. **18**: 201–205.

64. **Maletzki, C., Linnebacher, M., Kreikemeyer, B. and Emmrich, J.,** Pancreatic cancer regression by intratumoural injection of live Streptococcus pyogenes in a syngeneic mouse model. *Gut* 2008. **57**: 483–491.
65. **Linnebacher, M., Maletzki, C., Emmrich, J. and Kreikemeyer, B.,** Lysates of S. pyogenes serotype M49 induce pancreatic tumor growth delay by specific and unspecific antitumor immune responses. *J Immunother* 2008. **31**: 704–713.
66. **Matzinger, P.,** The danger model: a renewed sense of self. *Science* 2002. **296**: 301–305.
67. **Kanzler, H., Barrat, F. J., Hessel, E. M. and Coffman, R. L.,** Therapeutic targeting of innate immunity with Toll-like receptor agonists and antagonists. *Nat Med* 2007. **13**: 552–559.
68. **Wang, B. Z., Quan, F. S., Kang, S. M., Bozja, J., Skountzou, I. and Compans, R. W.,** Incorporation of membrane-anchored flagellin into influenza virus-like particles enhances the breadth of immune responses. *J Virol* 2008. **82**: 11813–11823.
69. **Hobohm, U., Stanford, J. L. and Grange, J. M.,** Pathogen-associated molecular pattern in cancer immunotherapy. *Crit Rev Immunol* 2008. **28**: 95–107.
70. **Napolitani, G., Rinaldi, A., Bertoni, F., Sallusto, F. and Lanzavecchia, A.,** Selected Toll-like receptor agonist combinations synergistically trigger a T helper type 1-polarizing program in dendritic cells. *Nat Immunol* 2005. **6**: 769–776.
71. **Yacyshyn, B. R., Barish, C., Goff, J., Dalke, D., Gaspari, M.,** *et al.* Dose ranging pharmacokinetic trial of high-dose alicaforsen (intercellular adhesion molecule-1 antisense oligodeoxynucleotide) (ISIS 2302) in active Crohn's disease. *Aliment Pharmacol Ther* 2002. **16**: 1761–1770.
72. **Bohach, G. A., Fast, D. J., Nelson, R. D. and Schlievert, P. M.,** Staphylococcal and streptococcal pyrogenic toxins involved in toxic shock syndrome and related illnesses. *Crit Rev Microbiol* 1990. **17**: 251–272.
73. **Cowdery, J. S., Chace, J. H., Yi, A. K. and Krieg, A. M.,** Bacterial DNA induces NK cells to produce IFN-gamma in vivo and increases the toxicity of lipopolysaccharides. *J Immunol* 1996. **156**: 4570–4575.
74. **Otto, F., Schmid, P., Mackensen, A., Wehr, U., Seiz, A.,** *et al.* Phase II trial of intravenous endotoxin in patients with colorectal and non-small cell lung cancer. *Eur J Cancer* 1996. **32A**: 1712–1718.
75. **Asprodites, N., Zheng, L., Geng, D., Velasco-Gonzalez, C., Sanchez-Perez, L. and Davila, E.,** Engagement of Toll-like receptor-2 on cytotoxic T-lymphocytes occurs in vivo and augments antitumor activity. *FASEB J* 2008. **22**: 3628–3637.
76. **Lorenz, E.,** TLR2 and TLR4 expression during bacterial infections. *Curr Pharm Des* 2006. **12**: 4185–4193.
77. **Loof, T. G., Goldmann, O. and Medina, E.,** Immune recognition of Streptococcus pyogenes by dendritic cells. *Infect Immun* 2008. **76**: 2785–2792.
78. National Cancer Institute Seer cancer statistics review. 2005. http://seer.cancer.gov/csr/1975_2004/results_merged/topic_historical_mort_trends.pdf
79. **Schlehofer, B., Blettner, M., Preston-Martin, S., Niehoff, D., Wahrendorf, J.,** *et al.* Role of medical history in brain tumour development. Results from the international adult brain tumour study. *Int J Cancer* 1999. **82**: 155–160.
80. **Ma, X., Buffler, P. A., Selvin, S., Matthay, K. K., Wiencke, J. K.,** *et al.* Daycare attendance and risk of childhood acute lymphoblastic leukaemia. *Br J Cancer* 2002. **86**: 1419–1424.
81. **Perrillat, F., Clavel, J., Auclerc, M. F., Baruchel, A., Leverger, G.,** *et al.* Day-care, early common infections and childhood acute leukaemia: a multicentre French case-control study. *Br J Cancer* 2002. **86**: 1064–1069.
82. **Deidier, A.** Dissertation Medecinal et Chirurgical sur les. Tumeurs, Paris 1725.
83. **Braunstein, A.,** Experimentelle und klinische Grundlagen fuer Malariabehandlung des Krebses. *Z Krebsforsch* 1929. **29**: 468–490.
84. **Sinek, F.,** Versuch einer statistischen Erfassung endogener Faktoren bei Carcinomerkrankungen. *Z Krebsforsch* 1936. **44**: 492–527.
85. **Remy, W., Hammerschmidt, K., Zänker, K. S., Ulm, K., Theisinger, W.,** *et al.* Tumorträger haben selten Infekte in der Anamnese. *Med Klin* 1983. **78**: 95–98.
86. **Ronne, T.,** Measles virus infection without rash in childhood is related to disease in adult life. *Lancet* 1985. **1**: 1–5.
87. **Grossarth-Maticek, R., Frentzel-Beyme, R., Kanazir, D., Jankovic, M. and Vetter, H.,** Reported herpes-virus-infection, fever and cancer incidence in a prospective study. *J Chronic Dis* 1987. **40**: 967–976.
88. **Abel, U., Becker, N., Angerer, R., Frentzel-Beyme, R., Kaufmann, M.,** *et al.* Common infections in the history of cancer patients and controls. *J Cancer Res Clin Oncol* 1991. **117**: 339–344.
89. **Devitt, C., Dernell, W., Jameson, V., Lafferty, M., Kuntz, C. and Powers, B.,** Effect of postoperative infection in dogs with osteosarcoma treated with limb-sparing surgery. Proceedings of the 16th Annual Conference of the Veterinary Cancer Society. 1996. pp. 104–105.
90. **Mastrangelo, G., Fadda, E. and Milan, G.,** Cancer increased after a reduction of infections in the first half of this century in Italy: etiologic and preventive implications. *Eur J Epidemiol* 1998. **14**: 749–754.
91. **Grufferman, S., Wang, H. H., DeLong, E. R., Kimm, S. Y., Delzell, E. S. and Falletta, J. M.,** Environmental factors in the etiology of rhabdomyosarcoma in childhood. *J Natl Cancer Inst* 1982. **68**: 107–113.
92. **Hoffmann, C., Rosenberger, A., Troger, W. and Buhring, M.,** Childhood diseases, infectious diseases, and fever as potential risk factors for cancer ? *Forsch Komplementarmed Klass Naturheilkd* 2002. **9**: 324–330.