Spontaneous regression of cancer: A therapeutic role for pyrogenic infections?

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Kok-Ho's interest lies in infectious diseases and oncology; in particular, the use of microbial therapeutics in cancer treatment. Kok-Ho's primary motivation in medicine is to find a novel therapy for cancers with poor prognosis so that cancer patients who are incurable now may potentially have a new leash of life in the near future.

Spontaneous regression of cancer is a phenomenon that is not well understood. While the mechanisms are unclear, it has been hypothesised that infections, fever and cancer are linked. Studies have shown that infections and fever may be involved in tumour regression and are associated with improved clinical outcomes. This article will examine the history, evidence and future prospects of pyrogenic infections towards explaining spontaneous regression and how they may be applied to future cancer treatments.

Introduction

Spontaneous regression of cancer is a phenomenon that has been observed since antiquity. [1] It can be defined as a reversal or reduction of tumour growth in instances where treatment has been lacking or ineffectual. [2] Little is known about its mechanism but two observations in cancer patients are of particular interest: first, infections have been shown to halt tumour progression while second, development of fever has been associated with improved prognosis.

Until recently, fever and infections have been regarded as detrimental states that should be minimized or prevented. However, in the era preceding the use of antibiotics and antipyretics, the prior observations were prevalent and were used as the basis of crude yet stunningly effective immunological-based treatments. The promise of translating that success to modern cancer treatment is a tempting one and should be examined further.

History: Spontaneous Regression & Coley's Toxins

Spontaneous regression of cancers was noted as early as the 13th century. The Italian Peregrine Lazoisi was afflicted with painful leg ulcers which later developed into a massive cancerous growth. [3]The growth broke through the skin and became badly infected. Miraculously, the infection induced a complete regression of the tumour and surgery was no longer required. He later became the patron saint of cancer sufferers.

Reports that associated infections and tumour regression continued to grow. In the 18th century, Trnka and Le Dran reported cases of breast cancer regressions which occurred after tumour site infection. [4, 5] These cases are often accompanied by signs of inflammation and fever and gangrene are common. [3]

In the 19th century, such observations became the basis of early clinical trials by physicians such as Tanchou and Cruveillhier. Although highly risky, they attempted to replicate the same conditions artificially by applying a septic dressing to the wound or injecting patients with pathogens such as malaria. [1] The results were often spectacular and suddenly, this rudimentary form of 'immunotherapy' seemed to offer a genuine alternative to surgery.

Until then, the only option for cancer was surgery and outcomes were at times very disappointing. Dr. William Coley (a 19th century New York surgeon) related his anguish after his patient died despite radical surgery to remove a sarcoma of the right hand. [3] Frustrated by the limitations of surgery, he sought an alternative form of treatment and came across the work of the medical pioneers Busch and Fehleisen. They had earlier experimented with erysipleas, injecting or physically

applying the causative pathogen, *Streptococcus pyogenes*, onto the tumour site. [6] This was often followed by a high fever which correlated with a concomitant decrease in tumour size in a number of patients. [3] Coley realized that using live pathogens was very risky and he eventually modified the approach using a mixture of killed *S. pyogenes* and *Serratia marescens*. [7] The latter potentiated the effects of *S. pyogenes* such that a febrile response can be induced safely without an 'infection', and this mixture became known as Coley's toxins. [1]

A retrospective study in 1999 showed that there was no significant difference in cancer death risk between patients treated using Coley's toxins and those treated with conventional therapies (i.e. chemotherapy, radiotherapy and surgery). [8] Data from the second group was obtained from the Surveillance Epidemiology End Result (SEER) registry in the 1980s. [3] This observation is remarkable given that Coley's toxins were developed at a fraction of the cost and resources afforded to current conventional therapies.

Researchers also realized that Coley's toxins have broad applicability and are effective across cancers of mesodermal embryonic origin such as sarcomas, lymphomas and carcinomas. [7] One study comparing the five-year survival rate of patients with either inoperable sarcomas or carcinomas found that those treated with Coley's toxin showed had a survival rate as high as 70-80%. [9]

Induction of a high grade fever proved crucial to the success of this method. Patients with inoperable sarcoma who were treated with Coley's toxins and developed a fever between 38-40 °C had a five-year survival rate three times higher than that of afebrile patients. [10] As cancer pain can be excruciating, pain relief is usually required. Upon administration of Coley's toxins, an immediate and profound analgesic effect was often observed; allowing the discontinuation of narcotics. [9]

Successes related to 'infection' based therapies are not isolated. In the early 20th century, Nobel laureate Dr. Julius Wagner-Jauregg used tertian malaria injections in the treatment of neurosyphilis-induced dementia paralytica. [3]This approach relied on the induction of prolonged and high grade fevers. Considering the high mortality rate of untreated patients in the pre-penicillin era, he was able to achieve an impressive remission rate of approximately one in two patients. [11]

More recently, Bacillus Calmette-Guérin (BCG) vaccine has been used in the treatment of superficial bladder cancers. [12] BCG consists of live attenuated *Mycobacterium bovis* and is commonly used in tuberculosis vaccinations. [12,13] Its anti-tumour effects are thought to involve a localized immune response stimulating production of inflammatory cytokines such as tumour necrosis factor α (TNF-α) and interferon *γ* (IFN-*γ*). [13] Similar to Coley's toxins, it uses a bacterial formulation and requires regular localized administration over a prolonged period. BCG is shown to reduce bladder cancer recurrence rates in nearly 70% of cases and recent clinical trials suggest a possible role in colorectal cancer treatment. [14] From these examples, we see that infections or immunizations can have broad and effective therapeutic profiles.

Opportunities Lost: The End of Coley's Toxins

After the early success of Coley's toxins, momentum was lost when Coley died in 1936. Emergence of chemotherapy and radiotherapy overshadowed its development while aseptic techniques gradually gained acceptance. After World War II, large-scale production of antibiotics and antipyretics also allowed better suppression of infections and fevers. [1] Opportunities for further clinical studies using Coley's toxins were lost when despite decades of use, it was classified as a new drug by the US Food and Drug Administration (FDA). [15] Tightening of regulations regarding clinical trials of new drugs after the thalidomide incidents in the 1960s meant that Coley's toxins were highly unlikely to pass the stringent safety requirements. [3]

With fewer infections, spontaneous regressions became less common. An estimated yearly average of over twenty cases in the 1960-80s decreased to less than ten cases in the 1990s. [16] It was gradually believed that the body's immune system had a negligible role in tumour regression and focus was placed on chemotherapy and radiotherapy. Despite initial promise, these therapies have not fulfilled their full potential and the treatment for certain cancers remains out of reach.

In a curious turn of events, advances in molecular engineering have now provided us with the tools to transform immunotherapy into a viable alternative. Coley's toxins have provided the foundations for early immunotherapeutic approaches and may potentially contribute significantly to the success of future immunotherapy.

Immunological Basis of Pyrogenic Infections

The most successful cases treated by Coley's toxins are attributed to: successful infection of the tumour, induction of a febrile response and daily intra-tumoural injections over a prolonged period.

Successful infection of tumour

Infection of tumour cells results in infiltration of lymphocytes and antigen-presenting cells (APCs) such as macrophages and dendritic cells (DCs). Binding of pathogen-associated molecular patterns (PAMPs) (e.g. lipopolysaccharides) to toll-like receptors (TLRs) on APCs induces activation and antigen presentation. The induction process also leads to the expression of important co-stimulatory molecules such as B7 and interleukin-12 (IL-12) required for optimal activation of B and T cells. [17] In some cases, pathogens such as the zoonotic vesicular stomatitis virus (VSV) have oncolytic properties and selectively lyse tumour cells to release antigens. [18]

Tumour regression or progression depends on the state of the immune system. A model of duality in which the immune system performs either a defensive or reparative role has been proposed. [1, 3] During the defensive mode, tumour regression occurs and immune cells are produced, activated and mobilized against the tumour. In the reparative model, tumour progression is favoured and invasiveness is promoted via immunosuppressive cytokines, growth factors, matrix metalloproteinases and angiogenesis factors. [1, 3]

The defensive mode may be activated by external stimuli during infections; this principle can be illustrated by the example of M1/M2 macrophages. M1 macrophages are involved in resistance against infections and tumours and produce pro-inflammatory cytokines such as IL-6, IL-12 and IL-23. [19, 20] M2 macrophages promote tumour progression and produce anti-inflammatory cytokines such as IL-10 and IL-13. [19, 20] M1 and M2 macrophage polarization is dependent on transcription factors such as interferon response factor 5 (IRF5). [21] Inflammatory stimuli such as bacterial lipopolysaccharides induce high levels of IRF5 and this commits macrophages to the M1 lineage while also inhibiting expression of M2 macrophage marker expression. [21] This two-fold effect may be instrumental in facilitating a defensive mode.

Induction of febrile response

In Matzinger's 'danger' hypothesis, the immune system responds to signals produced during distress known as danger signals, including inflammatory factors released from dying cells. [22] T cells remain anergic unless both danger signals and tumour antigens are provided. [23] A febrile response is advantageous as fever is thought to facilitate inflammatory factor production. Cancer cells are also more vulnerable to heat changes and elevated body temperature during fever may promote cell death and the massive release of tumour antigens. [24]

Besides a physical increase in temperature, fever encompasses profound physiological effects. An example of this is the induction of heat-shock protein (HSP) expression on tumour cells. [16] Studies have shown that Hsp70 expression on carcinoma cells promotes lysis by natural killer T (NKT) cells *in vitro*, while tumour expression of Hsp90 may play a key role in DC maturation. [25, 26] Interestingly, HSPs also associate with tumour peptides to form immunogenic complexes involved in NK cell activation. [25] This is important since NK cells help overcome subversive strategies by cancer cells to avoid T cell recognition. [27] Down regulation of major histocompatibility complex (MHC) expression on cancer cells results in increased susceptibility to NK cell attacks. [28] These observations show that fever is equally adept at stimulating innate and adaptive responses.

Route and duration of administration

The systemic circulation poses a number of obstacles for successful delivery of infectious agents to the tumour site. Neutralization by pre-immune Immunoglobulin M (IgM) antibodies and complement activation impede pathogens. [18] Infectious agents may bind non-specifically to red blood cells and undergo sequestration by the reticuloendothelial system. [29] In the liver, specialized macrophages called, Kupffer cells, can also be activated by pathogen-induced TLR binding and cause inflammatory liver damage. [29] An intratumoural route therefore has the advantage of circumventing most of these obstacles to increase the probability of successful infection. [18]

It is currently unclear if innate or adaptive immunity is predominantly responsible for tumour regression. Coley observed that shrinkage often occurred hours after administration whereas if daily injections were stopped, even for brief periods, the tumour continued to progress. [30] Innate immunity may therefore be important and this is consistent with insights from vaccine development, in which adjuvants enhance vaccine effectiveness by targeting innate immune cells via TLR activation. [1]

Although T cell numbers in tumour infiltrates are substantial, tolerance is pervasive and attempts to target specific antigens have been difficult due to antigenic drift and heterogeneity of the tumour microenvironment. [31] A possible explanation for the disproportionality between T cell numbers and the anti-tumour response is that the predominant adaptive immune responses are humoral rather than cell-mediated. [32] Clinical and animal studies have shown that spontaneous regressions in response to pathogens like malaria and *Aspergillus* are mainly antibody mediated. [3] Further research will be required to determine if this is the case for most infections.

Both innate and adaptive immunity are probably important at specific stages with sequential induction holding the key to tumour regression. In acute inflammation, innate immunity is usually activated optimally

and this in turn induces efficient adaptive responses. [33] Conversely, chronic inflammation involves a detrimental positive feedback loop that acts reversibly and over-activates innate immune cells. [34] Instability of these immune responses can result in suboptimal anti-tumour responses.

Non-immune considerations and constructing the full picture

Non-immune mechanisms may be partly responsible for tumour regression. Oestrogen is required for tumour progression in certain breast cancers and attempts to block its receptors by tamoxifen have proved successful. [35] It is likely that natural disturbances in hormone production may inhibit cancerous growth and promote regression in hormone dependent malignancies. [36]

Genetic instability has also been mentioned as a possible mechanism. In neuroblastoma patients, telomere shortening and low levels of telomerase have been associated with tumour regression. [37]This may be due to the fact that telomerase activity is required for cell immortality. Other potential considerations may include stress, hypoxia and apoptosis but these are not within the scope of this review. [38]

As non-immune factors tend to relate to specific subsets of cancers, they are unlikely to explain tumour regression as a whole. They may instead serve as secondary mechanisms which support a primary immunological system. During tumour progression, these non-immune factors may either malfunction or become the target of subversive strategies.

A simplified outline of the possible role of pyrogenic infections in tumour kinetics is illustrated below (Figure 1).

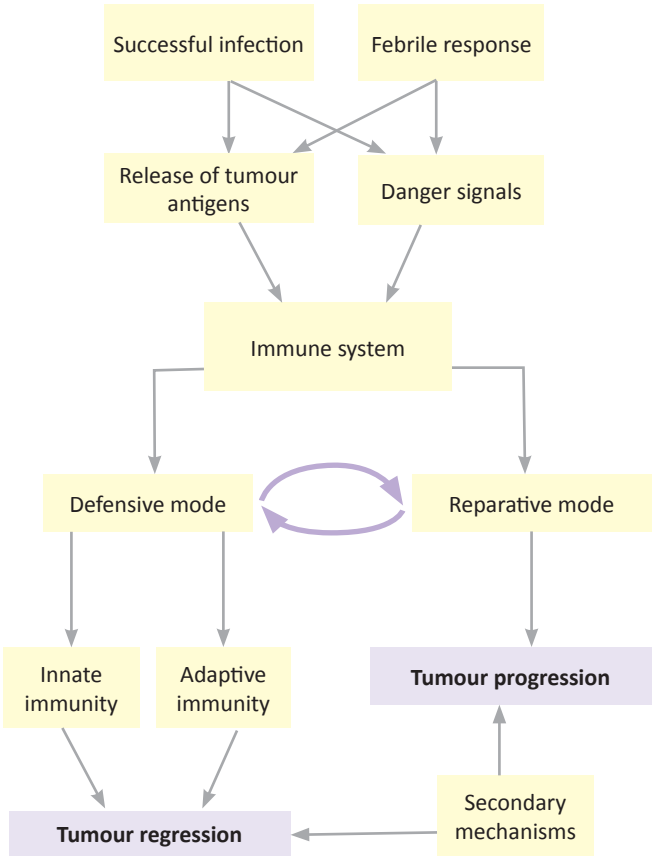

Figure 1. *Hypothetical role of pyrogenic infections in waxing and waning of tumours. Successful infection and febrile response promote release of tumour antigens and danger signals, tilting the immune system to a defensive mode where innate and adaptive responses favour tumour regression. Lack of these stimuli triggers the reparative mode and results in tumour progression. Secondary mechanisms may influence regression or progression in specific cancers.*

Discussion

The intimate link between infections, fever and spontaneous regression is slowly being recognized. While the incidence of spontaneous regression is steadily decreasing due to circumstances in the modern clinical setting, Coley's toxins are a timely reminder that lessons from the past can shape the future of cancer therapy.

Limitations to be addressed

Immunotherapy in its present form has been limited in efficacy primarily due to several reasons. Firstly, single cytokines or PAMPs have been used in trials in the hope of achieving an immediate effect. [39] This 'magic bullet' approach fails to recognize that a typical immune response involves a complex cascade of events, and that PAMPs may be involved in triggering several TLRs simultaneously. This is difficult to replicate given our incomplete understanding of intricate multi-faceted immune processes. Realistically, this may currently only be achieved by natural challenges such as infections.

Furthermore, the use of single cytokines and their related inhibitors remains a dilemma. This is best illustrated by the incorporation of recombinant TNF-α and anti-TNF-α agents into cancer treatment. TNF-α is produced physiologically by cancers to maintain a tumour-promoting chronic inflammatory state. [40, 41] A pronounced anti-tumour effect is observed when high therapeutic dosages of exogenous TNF-α are administered and transition to acute inflammation occurs. [41] However, this beneficial effect is often achieved at a risk of severe toxicities like organ failure. [40] Similarly, anti-TNF-α agents like infliximab (anti-TNF-α antibody) and etanercept (soluble TNF-α receptor) may reduce pathological levels of TNF-α but there is a trade-off between impeding tumour progression and higher risk of opportunistic infections (e.g. listeriosis) and possibly secondary malignancies (e.g. lymphoma) due to suppression of TNF-α protective effects. [41] These paradoxical observations suggest that the present form of cytokine-based immunotherapy is still fraught with difficulties.

Secondly, fever immunology has been largely neglected. Febrile responses are pushed aside as detrimental side effects; the potential benefits have been ignored. [6] Fever is important in potentiating immune responses, but the use of antipyretics alongside immunotherapy appears to defeat the purpose of stimulating the body's immune system.

Recent studies have started to demonstrate the prophylactic potential of pyrogenic infections. Koelmel *et al.* analyzed the melanoma risk in a group of more than six hundred patients and found that the lifetime risk is lowered to two in five patients if the frequency of infections and severity of fever are both increased. [42] This brings about an interesting dilemma, where we are caught between resolving current infections at a greater risk of developing cancer later in life. A change in treatment approach can be justified if this is proved for other cancers. It is foreseeable that such a change ultimately depends on our ability to discern between cancer-causing and beneficial infections and their associated inflammatory patterns (i.e. chronic or acute).

Some of Coley's techniques (i.e. intra-tumoural and prolonged administration) are currently favoured in immunotherapy, illustrating that some key principles remain useful over time. Nonetheless, certain technical difficulties will need to be resolved. An intra-tumoural route sometimes requires multiple injections to achieve a desired level of infection while prolonged administration and its long term discomfort may reduce treatment compliance and in turn, affect the clinical outcome.

Incorporating Coley's principles into current treatment regimes

In the near future, Coley's principles will need to coexist alongside current treatment modalities. This is because immunotherapy has yet to produce consistent clinical results to justify a mainstream role in cancer therapy and realistically, there is still some way to go before we

can fully comprehend and harness the potential of the immune system.

Theoretically, immunotherapy is based on stimulating the immune system while existing modalities such as chemotherapy and radiotherapy tend to suppress it. This explains why early clinical trials involving bacterial extracts called mixed bacterial vaccine (MBV) have not been as successful as predicted. [14] Selected patients have usually undergone conventional treatment previously and MBV is only given at a late stage of cancer development as a last resort. [43] Conditions then would have been predominantly immunosuppressive, severely affecting the ability of MBV to stimulate immunity.

However, recent clinical trials involving oncolytic viruses seem to suggest a role for immunosuppression in mediating an effective virus-mediated anti-tumour response. Chemotherapeutic drugs like cyclophosphamide can suppress antibody neutralization of viruses and facilitate delivery to tumour sites. [44] Similarly, a radiotherapy-reovirus combination has shown promising results in promoting T-cell trafficking and recognition of tumour cells. [16] It appears that the main determinant is not the theoretical nature of each treatment modality but rather, how they can be integrated to provide a synergistic effect. Furthermore, this also suggests that viruses may be more suitable for combinatorial treatments. If so, incorporating infection-based immunotherapy into cancer treatment is highly feasible once the correct combinations and infectious agents are identified.

Conclusion

As we grapple with the challenges and limitations of cancer treatment, it may prove beneficial to revisit the work of early experimenters such as William Coley. His contributions have been neglected for decades but as we begin to recognize the significance of his work, his status as a pioneer of cancer immunotherapy appears to be well justified.

Conflict of interest

None declared.

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