Commentary

The relevance of non-linear mathematics (chaos theory) to the treatment of cancer, the role of the immune response and the potential for vaccines

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Summary

Non-linear mathematics or ‘chaos’ theory was first applied in the prediction of complex systems such as the weather. Chaotic systems exhibit not only apparently random unpredictability, but also a degree of determinism, in that randomness remains confined within specific parameters. Biological systems manifest many of the features of chaotic systems, including the inherent repetition of self, fractal structure, and the existence of strange attractors. In this article, the interaction between a tumour and the immune system is examined with regard to the capacity for immunotherapy to influence these two complex systems beneficially. It is postulated that judicious immunization may lead to profound changes in the stability of this interaction, in the favour of the immune system’s ability to recognize tumour cells.

Introduction

The term ‘chaos theory’ suggests disorder and unpredictability, which is partially right, but does not represent the other very important aspect of chaotic systems—that they are deterministic, with changes occurring only within prescribed borders. Their unpredictability arises because minuscule changes in the starting conditions of a chaotic system can produce widely different outcomes. A good example of this is a simplified vertical pinball machine, where a ball is dropped and its path is interrupted by two rows of equally spaced pins, finally landing in one of 16 different pockets. The interaction with the first row of pins greatly amplifies any minor difference between one try and the next, and this is again amplified by the interaction with the next row. In practical terms, it is impossible to predict in which pocket the ball will come to rest, even if the greatest care is taken to reproduce the previous result (Figure 1). A more familiar example of this principle is the problem of weather forecasting. Very small changes in initial conditions can produce large changes in the weather, even in the short to medium term, making forecasting extremely difficult. Lorenz tried to solve this problem during WWII so that the weather could be more accurately predicted for air-force sorties. Working with a simple computer, he realized that repeated equations in which several decimal places were rounded up (e.g. 2.978658 becomes 2.97866) gave different results, leading to the conclusion that small changes in the initial conditions can lead to highly diverse outcomes. Lorenz predicted that if we had supercomputers (to handle all the decimal places) and means of obtaining accurate information, such as satellites, then we would be able to forecast the day-to-day weather much more accurately. The advent of super-
It is likely that the non-linear dynamics used to model weather systems are also applicable to biological systems. Biological systems are inherently variable, and need to be so to adapt to changing environments. Biological systems are also inherently lazy, in that if a formula works it will be re-iterated again and again rather than expending the energy involved in finding a new solution. This is seen repeatedly in biological systems from gross structural anatomy all the way through to basic developmental genes. In higher organisms, there are two major complex systems that interact with the environment. One is the nervous system, which receives the various senses, and the other is the immunological system, which identifies and repels dangerous micro-organisms. Both are exceedingly complex, interactive, and capable of memory. The immune system has many layers of function, from mucosal and innate immunity through to highly specific humoral and cell-mediated response recognition. It is 'set' to differentiate between self and non-self early on in life, and even though it produces hundreds of millions of T cells, it deletes the majority because they recognize self. Although the details of how this is achieved and how the resultant immune system matures and evolves as the body ages are far from clear, it is amazing that, given the similarity of many micro-organisms to self antigens, it is so effective in controlling microbiological organisms and yet so tolerant of self antigens. The consequence of this system when it fails are fortunately much more rare than theory would suggest, and are manifested in conditions such as rheumatoid arthritis, multiple sclerosis, diabetes, etc. It is therefore likely that the immune system focuses around strange attractors along the lines suggested by Lorenz for his weather systems. What these attractors are, we do not know, but there is increasing evidence that there is a focusing of the immune system based on minor differences in its own self make-up. Good examples of this are given by Ira Cohen and his theory that HLA and immunoglobulin act as a homunculus, in which he suggests that the immune system is focused on regions of HLA and immunoglobulin because these are the components that pathogenic micro-organisms have most often hijacked. Therefore the immune system is tuned to attack invading hosts bearing such sequences rapidly, and we do not have to wait weeks for the relevant immune response to be made. That this focused attack can be effective, and reduce a micro-organism load which is similar enough to self to cause an auto-immune attack, is demonstrated by auto-immune phenomena such as skin rashes, arthritis, etc. which accompany most acute micro-organism infections, albeit transiently.

The weather and the immune systems

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Figure 1. Cartoon of apparatus to demonstrate the unpredictability of a ball dropped from the same site and height and ending up in a specific hole, its route being altered at random by the first and/or second row of pins.

computers and satellites has enabled this prediction to come true, at least for short- to medium-term forecasting, Lorenz himself devised a computer and a program to perform some of these calculations, which form the basis of what is loosely known as chaos theory. It is important to appreciate that a lot of ‘chaos’ results from simple re-iteration of simple equations (Figure 2). Indeed, re-iterating some of these equations can lead to wonderful complex structures, such as the Mandelbrot set which, while ever diversifying at higher magnification, always appears to come back to its original shape, with many repetitions of recognizable forms or fractals visible at different levels of magnification (Figure 2). There is an inherent variability in these forms, many of which are similar to biological structures. It is the ability of chaos to form repeatable, recognizable structures on different scales, such as fractals, and also to return to previous starting points of forms, encompassing what Lorenz called the strange attractor, which provides the excitement in applying this approach to biological problems, especially cancer.

The limitation of variability in that it takes place within a certain wedge of possibilities, was first noted by the French mathematician Poincaré, who noted that the expression of a variable mathematical problem was always confined to a certain area or box. That chaotic variability has an inherent predictability can be seen in the weather. Although it is extremely difficult to predict the weather in 3 to 4 weeks, it is likely that in 12 months the weather will be roughly similar to now, and that the seasons will follow in a recognizable pattern of change.
Cohen claims that these features settle down following the fine tuning of immune responses to the HLA-like sequences, focusing on specific epitopes of the invading agent. This theory is basically a further embellishment of the role of HLA and immunoglobin in determining self from non-self.

**Th1 and Th2 responses**

Many other chronic diseases and conditions are associated with changes in the ratio of different antibodies to self-HLA class I and class II. Susal and colleagues have proposed a theory whereby the change in focus between the class I and class II type can exacerbate chronic disease conditions such as HIV infection. These changes appear to parallel another immunological status change in certain disease states known as the Th1-Th2 theory where Th1 represents cell-mediated responses associated with IL-2, γ interferon and IL-12, and Th2 represents humoral responses associated with IL-4, 5, 6 and 10. (Table 1) In certain chronic disease conditions, especially chronic infectious states or chronic autoimmune states, a marked depletion of Th1 cytokine production and an increase of Th2 dominance in the immune system would appear to occur (Table 2). Several authors have clearly associated the dominance of these two different measurable components of the immune system with disease, in that the induction of a Th1- or Th2-dominant immune response can significantly alter the disease phenotype; for instance, HIV and many other chronic infections are associated with a loss of Th1 responses and increases in Th2 responses, which occurs against the background of a collapsing immune system.

The bottom line regarding the application of chaos theory to immunology, and its use in the treatment of disease, is that if a complex system such as the immune system can be viewed as a self-referential network based around ‘strange attractors’, disease states may lead to a collapse of certain components of the immune network. Conversely, moving the ‘attractor’ might change the relationship of the immune system to the disease state, whether an invading organism or a tumour. It is highly likely that when diseases such as melanoma and renal cancer respond to a wide variety of agents such as BCG, interferon, interleukin II and cellular vaccination, that these non-specific stimulants are altering the immune response by acting on a ‘strange’ attractor. This activity is obviously dependent on the other variable states of the immune response, which would explain why the response rate to these therapies is consistently similar at around 20–30%, a figure similar to drug response rates in many hundreds of studies in solid tumours such as lung cancer. The possibility that the chances of some of these approaches working would be greatly increased if major immunological attractors could be ‘reset’, is clearly attractive.

The concept of resetting the immune system has been proposed previously by Jerne, who proposed the network theory of the immune system, and suggested that anti-idiotype antibodies maintained a stable immune system and could also be used as vaccines to ‘reset’ the response. If as suggested by Susal et al., the changing ratio of anti-class I to anti-class II antibodies is the causal event and not a surrogate marker, then anti-idiotype vaccinations based on HLA moieties may yet have a role as tumour immunotherapy.

**Current issues in the recognition of tumour antigens**

Tumour cell lines only arise when they evade a complex system of controls, which occur at several levels. With regards to immune recognition, it has been appreciated for a long time that the immune system may well eliminate cancers in very early stages by constant surveillance of abnormal mutations, etc. This was first suggested by Burnett as the immune surveillance theory of cancer, but has been controversial, mainly because of the relative absence of any increase in solid tumours in patients with a long-term immunosuppressive condition such as AIDS. The only tumours which are associated with AIDS are those which are associated with viruses, and which appear with increasing immune deficiency, e.g. EBV-‘driven’ lymphomas, HHV8-‘driven’ Kaposi’s sarcoma, and HPV-‘driven’ cervical cancer.

The fact that the immune system is important in controlling solid tumours comes from a number of different observations, the first of which was made by Festenstein and colleagues, that HLA expression on tumours is reduced and increasingly so with the progression of metastatic disease. Although a significant trend, this is not the case in all tumours or any one tumour type. More recent studies have shown that often the specific MHC/HLA allele is downregulated, and that this is the allele which presents the immunodominant component of a tumour antigen to the immune system. However, as our understanding of the immune system slowly increases, it has become apparent that there are many other layers at which the ‘escape’ can work.

MHC (HLA molecules in humans) present(s) peptides to the T-cell receptor, an interaction that can lead to either activation or paradoxically to anergy. The need for at least one other signal or co-stimulus explains this marked difference in activity. Such
Non-linear mathematics (chaos theory) and cancer

Table 1 Cytokines associated with cell-mediated (CM) and humoral (H) immune responses

<table>
<thead>
<tr>
<th>Cytokine (Th1)</th>
<th>Cytokine (Th2)</th>
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<tbody>
<tr>
<td>IL-2</td>
<td>IL-4</td>
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<tr>
<td>IFN-γ</td>
<td>IL-5</td>
</tr>
<tr>
<td>IL-12</td>
<td>IL-6</td>
</tr>
<tr>
<td>*(TNFα)</td>
<td>*(TNFα)</td>
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* TNFα appears to be a Th1 cytokine when Th1 cytokines are dominant, having anti-microbial, and anti-tumour properties, yet is pathogenic when a systemic Th2 response is dominant. Nevertheless, in conditions associated with Th1 and organ-immune damage such as rheumatoid arthritis or multiple sclerosis, inhibiting TNFα can greatly ameliorate clinical disease.

Table 2 Diseases in which Th1 responses are depressed with relative enhancement of Th2 responses

<table>
<thead>
<tr>
<th>Disease</th>
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<tbody>
<tr>
<td>Chronic infectious diseases</td>
</tr>
<tr>
<td>HIV and AIDS</td>
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<tr>
<td>Leprosy (lepromatous)</td>
</tr>
<tr>
<td>Tuberculosis</td>
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<tr>
<td>Leishmaniasis</td>
</tr>
<tr>
<td>Malignancy</td>
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<tr>
<td>Melanoma</td>
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<tr>
<td>Prostate cancer</td>
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<tr>
<td>Colorectal cancer</td>
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<tr>
<td>Lymphomas</td>
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<tr>
<td>Myeloma</td>
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</tbody>
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are now recognized to be deficient in costimulatory and/or normal adhesion molecules.12

The immune system is complex, and even these two scenarios would be unlikely to evolve fast enough to escape immune control, and thus other mechanisms appear to be used by tumour cells to avoid elimination. Many tumours appear to secrete cytokines and other messages, which may interfere with their recognition by the cell-mediated arm of the immune response, especially humoral cytokines such as IL-6 and IL-10 which downregulate cell-

Figure 2. The famous Mandelbrot set (a) derived from iteration of an equation plotted in 2D. Ever-increasing magnification gives rise to complex patterns which have their analogies in nature (b, c). Note the recurrent appearance of the fractal of a within these ‘sets’.
mediated responses. These properties are probably only the tip of the iceberg, but their importance can be demonstrated in animal tumour model experiments in which poorly immunogenic cells can be rendered more immunogenic by transfecting cytokine or stimulatory molecule genes that will induce an immune response (where the wild-type tumour will not), yet the immune response may be sufficiently strong to recognize the wild-type tumour. Such strategies, which have been widely published, include the expression of HLA on the tumour cell and its use as a vaccine to enhance the presentation or tumour antigens, as well as the expression by genetic transfer, the addition of co-stimulatory signals such as B7-1, and cytokines, e.g. interleukin 2 and γ interferon. However, in some animal models, the cytokines which render tumour cells immunogenic are not confined to those associated with Th1, as IL-4 and IL-10 can also induce anti-tumour responses to the wild-type tumour cell.\(^\text{13}\)

These experiments all provide evidence that poorly immunogenic cells can be recognized by the immune system when their antigens are presented by a more effective antigen-presenting cell. This has been taken further by the use of ‘professional’ antigen-presenting cells (APC) such as dendritic cells (DC) to induce strong anti-tumour immune responses against tumour-cell antigens.\(^\text{14}\) In early experiments, tumour cells were used as a vaccine themselves without really knowing the identity of any specific antigens. A very large effort is now underway identifying libraries of tumour-cell antigens by CTLs, and more recently by serological analysis. This has led to the identification of a whole family of new antigens, such as MAGE and MART, which were both originally identified from cytotoxic T-cell lymphocytes from melanoma patients. Good responses against these antigens are in some cases associated with regression.\(^\text{15}\) There is therefore a considerable body of evidence that the immune system can control ‘non-immunogenic’ tumours, at least in laboratory animals. However, in the human clinical setting, it has been observed for a long time that a variety of immune stimulations, particularly with whole-cell vaccines with adjuvants such as BCG, can be helpful, as described by Morton and his colleagues for melanoma, who not only claim a 2–3 fold increase in survival but also an objective effect on established melanoma disease.\(^\text{16}\) Similar observations have been made with a variety of immunotherapies. More dramatic effects have been seen in solid tumours such as lung cancer, with the induction of anti-neuronal antibodies following a transfusion with cross-reactive antibodies, eliminating this established tumour.\(^\text{17}\) Many other anecdotal reports exist of non-specific immune stimulation leading to responses in solid tumours such as ovarian cancer.\(^\text{18,19}\)

**Cancer and chaos**

Living systems are non-linear, with infinite variation existing within given parameters. Non-linearity is probably essential for human development, as the number of neural interactions alone greatly exceeds (by 2 logs) the number of genes encoding the nervous system. Like the nervous system, the immune system is not set in stone but learns and imprints its environment. If these were linear systems, then clones would be the normal state of affairs! Non-linearity would also appear essential for rapid adaptation in other organ systems, such as the cardiovascular system. Although the heart was initially thought to be absolutely regular, speeding up or slowing gradually depending on oxygen command, it is now recognized that the only time the heart beats with absolute regularity is just before death. At such a time, it has lost the variability to respond. An analogy is the use of unstable aerodynamics in the new generation of fighter aircraft, where unless their instability is continually controlled by computer the aircraft crashes; the advantage however is that such unstable aircraft are much more manoeuvrable than stable aircraft, whose predictable dynamics also make them much easier targets for missiles.

**Cancer and heterogeneity—chaos?**

Biological systems are essentially non-linear, and with much of the ‘phenotype’ dependent on the environment or other factors, it is becoming increasingly clear that not everything can be predicted from a genome sequence. Genes do not have enough power to encode everything, and much is left to controlled random integration of cells and systems. For example, there is no need to encode the fingerprint pattern in the gene, as long as the skin is rough for gripping (identical twins do not necessarily have identical fingerprints) and the same goes for small blood vessels and pulmonary airways. Such architecture is built at the local level by the interaction of activating and inhibitory growth factors. As long as the tissue is built within the prescribed space and function there is no need to detail all the interactions. Such replicative similarity is hence fractal, and the fractal nature of the tracheal bronchial tree of blood vessel supply is obvious (Figure 3).

Having established the necessity of ‘chaotic’ systems for life, cancer is loss of control of a ‘normal’ chaotic system resulting in further ‘chaos’. Cancer is therefore by definition a state of genetic instability, whose effects at the molecular level raise the possibility of specific therapeutic drugs aimed at such areas of instability, as investigated by Vogelstein’s group.\(^\text{20}\) This is the crucial question: can unstable pathways
or aberrant gene expression (or loss of chromosomes and gene expression) be targeted to produce new therapies? One initial reaction is that targeting a specific apoptotic pathway or similar predicted Achilles' heel will only lead to the evolution of the instability in many cases, using alternative pathways as escape routes, and this is the fundamental reason why so many standard therapies are limited by low response routes and high relapse rates. The essence of this article is that such instability can be used to alter the ambit (or orbit) of the tumour, allowing inherent more complex biological systems to contain its growth. What is the precedent?

In 1985, NASA launched a satellite, hoping to intercept the comet Giacobini/Zinner. The satellite, known as the 3rd Interplanetary Communication Explorer (ICE/ICE/3), was equipped with small rockets from which hydrazine fuel was released in order to make the necessary corrections to place it in the correct orbit. Unfortunately, nearly all the fuel was used up in the initial launch procedure, rendering it unable to propel itself into the appropriate orbit to intercept the comet. Undeterred, groups of non-linear mathematicians calculated that the satellite could be nudged using a small hydrazine burn into an unstable orbit from where it could be 'nudged' again into another unstable orbit. Repeated five times, this allowed the successful interception with the comet. Similar procedures enabling interception millions of miles away have subsequently been used with the Vega, Gioti and Galileo satellites, the last of which successfully explored Jupiter.21

Understanding the chaotic or unstable mathematics of cancer and its interplay with the immune system may enable similar strategies to be employed in the treatment of cancer.

Cancer and the immune system

There would appear to be an almost innate belief that stimulating the immune system should be capable of controlling cancer, as it is hard to identify the first person to seriously try this approach as treatment. Eighteenth century French practitioners refer to the concept as both therapeutic and preventative, using other people's tumours, and it would not be surprising if it was entertained by other civilizations.

The first scientific application of this approach was that of William Coley, a New York surgeon at the turn of the century who besides pioneering immunotherapy was also the first surgeon in New York to obtain a 'radiotherapy machine'. Following his observation that survivors of postoperative septicaemia occasionally experienced regression of their residual tumour, he set out to make a septicaemic culture to mimic this process. Unfortunately, antibiotics were not yet invented and he merely substituted death by cancer for death by iatrogenic septicaemia. He therefore moved on to bacterial cell-wall preparations with which he was to work and improve on for the next 20 years or so. Initially vilified by his peers, who failed to reproduce his work with half-hearted attempts, Coley died having received widespread approbation, including from the Royal College of Surgeons.22 Unfortunately his work did not survive the impact of new radio and chemotherapy developments. Nevertheless, his experience developed the fundamentals, which are as appropriate today with regards to the development of 'immunotherapy' such as cancer vaccines and gene-therapy-based strategies. Briefly, he showed that the original preparation was crucial (quality control), as was the dose and administration schedule, as he discovered that the toxins had to be given repeatedly for at least 6 months to contain residual disease. It is likely that most of his colleagues, who rapidly disbanded this approach, used substandard schedules that were unlikely to be effective.

How did Coley's toxins induce such marked responses (and very long-term survivors) in patients with such lethal conditions as head and neck cancers, sarcomas and metastatic melanoma? Modern-day assessment of Coley's toxins show that they induce a marked cytokine release, involving mainly \( \alpha \)TNF and \( \gamma \)IFN. However, neither of these cytokines given alone is likely to induce tumour regression in the human situation, in stark contrast to the case of TNF in mice.

Most immunotherapy approaches other than Coley's toxins tried this century are either passive such as monoclonal antibodies, or active, in that they require a responding immune system in order to achieve their goal, such as vaccines. It is the latter that is more likely to lead to an understanding of how the 'whole' immune response acts against cancer cells.

Vaccines

The most basic approach to cancer vaccines is to use autologous tumour cells as the vaccine prepara-
tion, and if they are not available, allogeneic tumour cells. Several waves of interest have occurred in this approach over the last 90 years, the most recent revival is in the guise of gene therapy (GT), where autologous cells are transfected with cytokine genes such as interleukin-2 and given as a vaccine. The unfortunate transfer of allogeneic cancer cells, which although given as a vaccine, occasionally grew in the host, thus giving the host a second malignancy, marred early studies. The use of radiotherapy to sterilize allogeneic cells has fortunately eliminated this problem.

Nearly all early gene therapy approaches to treat cancer were in fact just ‘hi-tech’ cancer vaccines. Most of these schedules involved the genetic transfer and expression of genes encoding cytokines or co-stimulatory molecules into tumour cells to make them more immunogenic. The even more direct use of gene therapy to kill cancer cells, using retroviruses to target cancer cells and insert an enzyme which converts a benign prodrug into an active toxin, e.g. phosphorylating Ganciclovir, appears to induce a significant bystander effect which is immunologically mediated. Nevertheless, the similitude between the approaches appears to have escaped the major cancer funding agents (at least in the UK) who were busily closing down their Immunology departments with one hand, whilst trying to recruit molecular biologists to develop GT on the other. Needless to say this was an impressively expensive exercise in proving that the one thing we need to understand before engaging in high-tech GT is the immune system, which even if it is not being recruited against the cancer will almost certainly ‘take out’ the virus vector, particularly if it is adenovirus-based.

The history of the development of cancer vaccines is too large to repeat here, but suffice to say that it is replete with impressive anecdotes and unimpressive larger trials, especially if randomized. However, like Coley’s toxins, attention to detail distinguishes those with better apparent successes than those with no significant responses. The cell line(s) used, the number of cells used and the administration schedule, especially the frequency, are again very important criteria in determining response, and especially the adjuvants used, as they are closely linked with non-specific immune stimulation (for review see reference 24).

**Non-specific immunotherapy**

Cells have been used on the presumption that they would present tumour antigens to the immune system even though there was very little concept of what tumour antigens were. The major immunotherapies that have made an impact to date (albeit limited) are all non-specific such as BCG, interferon and interleukin-2.

BCG (the tuberculosis vaccine) was used as a treatment in its own right for a variety of conditions, especially melanoma. The most dramatic results were obtained when the inoculum was given directly into the lesions. Superficial lesions would necrose and sometimes disappear completely. Unfortunately, similar lesions not injected would not respond, indicating a local response not inducing memory to any shared antigen. Although melanoma is notorious for responding differentially, the lack of such bystander responses strongly suggests that systemic immunity was not being primed in an antigen-specific manner. Nevertheless, BCG was able to induce considerable improvement in a number of patients, which although individually impressive, were never enough when examined in a randomized trial setting. Moreover, BCG has established itself as a highly successful therapy for bladder cancer when given intravesically. Its role in vaccine studies is now that of an adjuvant having been used with the GM2 active toxin, e.g. phosphorylating Ganciclovir, when examined in a randomized trial setting.

Interferon was identified as an antiviral agent in the 1950s. There are three main types: alpha, beta and gamma. Only the first is relevant to cancer therapy. Initial studies used very high doses and resulted in considerable toxicity. The treatment was given intravenously and resulted in some improvements to a number of cancers. However, the high doses involved were very impractical in the absence of a reliable response. Lower doses were then tried in both treatments, as well as in an adjuvant setting, for a number of different tumours. The first tumour to show a marked response was the relatively rare hairy-cell leukaemia, which appeared to differentiate in the presence of interferon and become more susceptible to chemotherapy. Used as an adjuvant, it has been shown to increase survival in multiple myeloma. Its role in melanoma remains unresolved, in spite of optimistic early studies. Interferon also has activity in renal-cell cancer, with an activity of between 10 and 20%, being more effective in patients who have undergone a nephrectomy first. Initial trials claiming effectiveness in colorectal cancer when added to 5-fluorouracil in an adjuvant setting have not been reproduced.

Interleukin-2 had a very similar start to interferon, with claims that it was very effective when given at high doses particularly with ex vivo expanded lymphokine activated killer cells (LAK). Although the treatment was toxic and associated with deaths, some patients with melanoma, renal-cell cancer or lymphoma went on to have prolonged remissions. A
number of randomized studies confirmed such responses in a smaller percentage of patients. This figure did not alter dramatically when the ex vivo expansion was omitted, and when lower doses of IL-2 were tried. IL-2 is now licensed for renal-cell cancer, and is being used as a low-dose adjuvant in a small number of studies. As from the beginning of 1998, it has been licensed for melanoma as well. However, further applications can be anticipated as its role as an adjuvant or co-stimulatory messenger is appreciated. Direct injection into draining lymph nodes leads to marked softening and occasional regression of breast cancer, and it is surprising that this approach has not been more actively pursued given the interest in vaccines for most solid tumours including breast cancer.27

Antigen-specific vaccine approaches

Exactly what constitutes a tumour antigen has been the source of many debates over the last two decades or so. The nomenclature includes tumour-associated differentiation antigens (TADA), tumour-specific antigens (TSA), as well as tumour-associated antigens (TAA). TADA and TAA antigens are often oncofetal antigens which are expressed in the fetus and not in normal adult tissue, but can occur in tumours. Examples include carcinoma embryonic antigen (CEA) and alpha fetoprotein (AFP). Other antigens identified more recently include the MAGE and related families, which are present on tumour cells and are present, but not expressed or presented, in some normal tissues. Other antigens are apparently tumour-specific such as MART, and oncogene mutations such as ras 12, which is present in the majority of pancreatic cancer cells. Viral antigens associated with tumour cells are also potential TSAs, especially considering the number of human tumours associated with oncogenic viruses: cervical cancer with papilloma viruses, nasopharyngeal and Burkitt's lymphoma with Epstein Barr virus, and hepatoma with hepatitis B virus. More recently, human retroviruses have been linked to adult T-cell leukaemia-lymphoma (HTLV-1) and AIDS, which is associated with lymphomas and Kaposi's sarcoma (KS), is caused by HIV. However the specific cause of KS appears to be a new herpes virus (HHV-8) which may be activated by HIV. It is unclear whether these viral antigens will allow for the development of treatment based on vaccines. Nevertheless, a successful vaccine against HBV already exists and is responsible for a marked decline in the incidence of HBV infection and hence future hepatomas, and can therefore already claim in some sense to be an effective anti-cancer vaccine.28 There is a tremendous flurry of activity with regards the development of HPV and EBV vaccines and clinical trials have already commenced with some success albeit anecdotal, claimed for a therapeutic HPV vaccine.

The search for tumour-specific antigens has intensified with the advent of new technologies. Boon and colleagues29 have used an elaborate mechanism of using CTLs from patients with cancers which have undergone regression to identify the fragments of tumour expressed as a DNA library. The positive fragments are then analysed further with mass spectrometry and peptide identification to reduce the target to a small peptide sequence, which therefore is dependent on HLA restriction. Members of the melanoma antigen family (MAGE), were the first to be identified and have subsequently been followed by a number of similar antigens such as PAGE from prostate cancer.

In addition to these specific peptides, it should not be forgotten that many tumour antigens are not necessarily peptides, but include gangliosides as previously mentioned as well as lipid-based antigens. Other antigens are associated with complex structures such as mucin and tumour-specific epitopes such as MUC-1 have been described in a number of tumours and are already being targeted for therapeutic purposes.

Presentation of antigens, camouflage and decoys

The major components of antigen presentation include the antigen-presenting cell with either MHC class I or II on its surface, presenting an antigen as a peptide to the T-cell receptor on CD8 and CD4 cells, respectively (Figure 4). A major problem with this model is that it has been described as being able to induce not only antigen-specific proliferation but also actively induce anergy to the same antigen. The discovery of co-stimulatory molecules such as B7-1 and B7-2, which present to associated molecules such as CD28 on the T cell, explained this conundrum, as the absence of the co-stimulatory signal results in anergy. It is therefore not surprising that defects in this pathway are common amongst tumours, which if they do not down-regulate their MHC molecules or alleles, downregulate their costimulatory molecules. This is just the beginning of their ability to effectively evade immune control, and hence strongly supports that, contrary to previous dogma, the immune response probably does play a major role in immune surveillance as postulated by Burnett. Other mechanisms used to evade an immune response include switching on anti-apoptotic pathways by such mechanisms as up-regulation of c-myc and other genes, expressing the fas ligand so that the incoming lymphocytes are apoptosed by a signal from the tumour, and the secretion of locally-acting immunosuppressive molecules such as the hum-
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autologous because of the need for haplotype restriction. However, a number of studies, including those of our own laboratory, strongly suggest that allogeneic cells can serve just as well with regards to a source of tumour antigens, as the cells are digested and the antigens presented by the host's dendritic cells in a haplotype-restricted manner. This is very encouraging for the development of multivalent vaccines, as autologous vaccines are restricted with regards to their practical application, due to the difficulty in obtaining enough tumour and establishing a cell line in many cases. Indeed, only melanoma constitutes a readily accessible supply of cells that can be grown in a cell line in as many as 50% of attempted cases. Even then, the patients are often dead by the time enough cells have been obtained for a vaccine or to transfect with immunostimulating genes, a process which is often labelled as gene therapy. Allogeneic vaccines have an added theoretical advantage is that they may be able to induce a graft-versus-host-like response. Such a response may have practical application, in that patients with leukaemia who have an allogeneic marrow transplant and develop a graft versus host disease have a better survival than those that do not. Moreover, a graft versus leukaemia activity can be separated from GVH in these patients. It is possible that allogeneic cells can induce a graft versus tumour response in some cancers.

Figure 4. Professional antigen-presenting cells (APCs) present antigen on MHC class II molecules to CD8 lymphocytes. Professional APCs also express costimulatory molecules which are necessary to induce an immune response. Many tumours do not express these molecules and induce anergy or non-responsiveness instead which is the pathway activated in the absence of a second signal. (MHC = HLA in tumours).

Orally-based cytokines, e.g. IL-10, IL-4. In addition to the foregoing there is also some evidence that the signalling mechanisms of otherwise normal lymphocytes are inhibited in cancer patients, by a mechanism which is unclear.

It is therefore no surprise that the antigen presentation needs to be a top priority for cancer vaccines in order to overcome the generalized lethargy of the immune system and the tumour-specific anergy. This explains why powerful adjuvants are needed and why the stimulus must be given at regular intervals, in contrast to infectious-disease-based vaccines.

With a growing body of evidence suggesting that an effective anti-tumour response needs to be able to enhance the cell-mediated or Th1 cytokine profile, this may explain the relative lack of efficacy of earlier studies using either no adjuvants at all or inappropriate ones such as alum. Even BCG, a popular adjuvant for cell-based vaccines, does not always stimulate the same immune response in different populations, which may explain its failure to act as a universal TB vaccine throughout the world. It appears that its response is determined by other factors such as the prior setting of the immune response with regards the dominance of a Th1 or Th2 profile. The ‘professional’ antigen-presenting cell would appear to be the dendritic cell, which needs activation and maturation for optimal function. Trials designed to maximize its potential are now underway in the clinic using in vitro expanded dendritic cells pulsed with tumour peptides before being used as a vaccine.

Whole-cell-based vaccines have tended to be autologous because of the need for haplotype restriction. However, a number of studies, including those of our own laboratory, strongly suggest that allogeneic cells can serve just as well with regards to a source of tumour antigens, as the cells are digested and the antigens presented by the host's dendritic cells in a haplotype-restricted manner. This is very encouraging for the development of multivalent vaccines, as autologous vaccines are restricted with regards to their practical application, due to the difficulty in obtaining enough tumour and establishing a cell line in many cases. Indeed, only melanoma constitutes a readily accessible supply of cells that can be grown in a cell line in as many as 50% of attempted cases. Even then, the patients are often dead by the time enough cells have been obtained for a vaccine or to transfect with immunostimulating genes, a process which is often labelled as gene therapy. Allogeneic vaccines have an added theoretical advantage is that they may be able to induce a graft-versus-host-like response. Such a response may have practical application, in that patients with leukaemia who have an allogeneic marrow transplant and develop a graft versus host disease have a better survival than those that do not. Moreover, a graft versus leukaemia activity can be separated from GVH in these patients. It is possible that allogeneic cells can induce a graft versus tumour response in some cancers.

Another major issue is whether or not the most effective response results from a class-I-induced CTL response, or whether a class II response is necessary instead, or as well. Thus it is essential to establish correlates of protection in both animal models and in human disease. Another issue not touched on previously here is the role of Natural Killer (NK) cells, which are capable of killing MHC-negative cells which have thus evaded MHC restricted killing. NK cells are stimulated by IL-12, which was originally known as NK growth factor, and hence it is likely that NK cells may play an important role in cell-mediated or Th1-like responses. It is also important to note that the most important anti-tumour response may require an immune component not yet discovered or poorly understood, and that more than one aspect of the immune response is required for an effective response. The complexity of the immune response to an aberrant self cell cannot be overstated. The fact that tumours express self antigens to which the immune system is tolerant shows that they have mastered the art of decoy. However, there is cause for optimism in the development of tumour vaccines, as that responses to some of these antigens can be to distinguish an antigen on a tumour from the same antigen on a normal cell.

It is proposed that the nature of the non-specific immune stimulation, like the importance of starting
conditions in chaos theory, determines large changes in the outcome. That small changes in key cytokines can induce marked clinical changes in clinical conditions has already been demonstrated using inhibitors of TNF-α such as antibodies or thalidomide, which can lead to marked clinical improvements in severe rheumatoid arthritis, aphthous ulcers, Behçet's disease and Crohn's disease, to name but a few, in a matter of days.

Many cancers, in common with a number of infectious diseases, appear to have lost cell-mediated immunity which can only be measured by the induction of IL-2 and γIFN by PMA-stimulated whole blood. In a study looking at patients with colorectal cancer, we were able to demonstrate a marked reduction in cytokines associated with monocyte cell stimulation in patients with all grades of tumour, even early Duke's A and B tumours. Upon removal of the tumour, this function (measured in the peripheral blood!) reverts to normal in the majority of cases with the exception of some patients with Duke's C or advanced disease that has split outside the colon wall. It is not yet known whether these patients have a higher chance of relapsing in the near future. (Heriot et al. submitted)

In patients with melanoma in which the Th1 or cell-mediated cytokine production can be restored with vaccination, complete and partial responses of established disease have been witnessed, as the balance between Th2 and Th1 cytokines is reversed in favour of the latter. Survival would also appear to be significantly improved in stage 4 patients with malignant melanoma treated with *Mycobacterium vaccae* (a relative of BCG) which induces a Th1 cytokine response in approximately a third of those treated. A similar benefit has been seen in prostate
cancer patients. (Maraveyas et al. submitted, and reference 35)

The Th1/Th2 axis—the immune system’s great attractors?

It is therefore proposed that the balance between Th1 and Th2 represents a stable state of joint or ‘strange’ attractors for the immune system and that disease upsets this balance. Moreover, it is proposed that correcting this imbalance can lead to at least an improvement in the clinical phenotype of disease. (Figure 5)

The interplay between a tumour and the immune response is so crucial to the development of cancer that it is also proposed that until the tumour escapes immune surveillance control, any mutation or loss of suppressor genes is of little consequence. It is of note that there appears to be a relationship with loss of Th1-dominant immune responses and the induction of angiogenesis, another property required for tumour growth and metastatic spread. This control of the Th1/Th2 imbalance may have major effects on tumour outcome by affecting other growth pathways such as angiogenesis. It is also likely that immune imbalance restoration may allow other more conventional therapies to be more predictably effective. This would appear to be the case for radiotherapy as well as likely for chemotherapy in the treatment of malignant melanoma.

Conclusion

The concept of anarchic complexity (cancer) interacting with a highly complex network immune system which can be described in terms of self-similarity (fractals), is so relevant to cancer and its integration within a host, that the implications with regards to new approaches to managing treatment cannot be ignored. Already the use of fractals to diagnose early tumours both at the histological and the imaging level (NMR) is having a major impact on our understanding of how cancer develops. The new approaches to treatment revealed by this understanding will hopefully not take many years to translate into clinical efficacy.

References


