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Review

Immunotherapy for malignant melanoma – Tracing Ariadne's thread through the labyrinth

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ARTICLE INFO

Article history:
Received 22 February 2009
Received in revised form 28 April 2009
Accepted 1 May 2009
Available online 3 June 2009

Keywords:
Melanoma
Immune surveillance
Immunotherapy
Toll-like receptors
Mycobacterium vaccae
Endogenous retrovirus
Regulatory T cells
Immune tolerance

ABSTRACT

A working group (FEBIM) within the European Organisation for Research and Treatment of Cancer undertook extensive studies on the possible association of infectious diseases and the risk of malignant melanoma. These studies provided evidence that several infectious diseases and also some vaccines including the anti-tuberculosis vaccine, BCG, derived from *Mycobacterium bovis*, confer a significant level of protection against this form of cancer. In recent years, the importance of immunoregulatory networks in the establishment of tolerance to tumour antigens and the key role of the innate immune system in the development of such networks have been recognised. The molecular patterns of micro-organisms activate pattern recognition receptors on antigen presenting cells and determine the qualitative nature of the ensuing immune response. Bacteria in the actinomycetales family, notably members of the genus *Mycobacterium*, exhibit particularly powerful adjuvant activity and profoundly affect underlying patterns of immune reactivity. In particular, there is growing evidence that a heat-killed preparation of a strain of *Mycobacterium vaccae* is able to down-regulate patterns of immune reactivity that favour the tumour and to induce those that lead to anti-cancer immune responses. The results of preliminary clinical observations with melanoma patients, and published studies on other cancers, point to the need for more formal clinical trials.

1. Introduction

Cutaneous malignant melanoma is an enigma. In many countries, the incidence of malignant of this form of cancer is increasing at a faster rate than any other neoplasm except lung cancer in women.[1–4] On the other hand, it is one of the few cancers in which spontaneous immune-mediated regressions have been observed and which have inspired many attempts to develop immunotherapeutic strategies, yet consistent success with this approach has been tantalisingly elusive. A melanoma, like any other tumour, is not a homogeneous structure but may be regarded as a complex-evolved tissue, having passed through a number of 'evolutionary' stages, not unlike Darwinian selection, driven largely by immune responses of the host. As a result of 'immunoediting' and 'tumour sculpting', tumours pass through the 'three Es'

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doi:10.1016/j.ejca.2009.05.002

– Elimination, Equilibrium and Escape,[5,6] and the clinically evident disease is therefore resistant to the diverse immune defenses of the host. Nevertheless, the occasional naturally occurring regressions and immunotherapeutic successes indicate that melanoma has an Achilles' heel and the challenge is to delineate and utilise this to the advantage of the patient.

In order to shed light on this Achilles' heel and to develop effective ways of exploiting it therapeutically, it is necessary to attempt to unravel several tangled threads. As microbiologists, the authors hope to address this challenge from a novel perspective.

2. Immunological aspects of the natural history of melanoma

Although there is considerable evidence that a key causative factor of cutaneous melanoma is exposure to light, especially ultraviolet light, the relationship is not straightforward, and other, as yet unknown, factors may play major roles in the changing trends in incidence.[7,8] The use of a mathematical model led to the conclusion that the rise in the incidence of melanoma, and possibly certain other cancers, that commenced in Sweden in the middle of the 20th century is attributable to a reduced efficiency of mechanisms that repair malignantly transformed cells.[9] Although it was assumed that this reduced efficiency affected intracellular DNA repair mechanisms which could have been compromised by exogenous factors such as increasing exposure to electromagnetic radiation, a hygiene-related defect in the regulation of the immune system has been postulated as an alternative plausible mechanism.[10]

The ability of severe infections to lead to regressions of tumours has been recognised since the middle of the 19th century,[11] and attempts were made, particularly by William Coley in the USA, to use extracts, subsequently termed Coley toxins, of bacteria causing erysipelas for therapy with reported success, particularly in tumours of mesodermal origin including melanoma.[12] In view of these and subsequent observations of beneficial effects of fever on melanoma, the Febrile Infections and Melanoma (FEBIM) group of the European Organisation for Research and Treatment of Cancer (EORTC) was established to conduct epidemiological studies in several European countries and Israel. The FEBIM group established that a history of severe, but increasingly uncommon, infections with fever >38.5 °C, including sepsis, pneumonia, pulmonary tuberculosis and *Staphylococcus aureus* infection, was associated with protection against melanoma.[13] Moreover, in patients with histories of severe infections the level of protection was directly related to the number of such infections. Thus those with histories of 1, 2–3 and 4 or more infections had odds ratios of 0.66, 0.63 and 0.32, with the trend being statistically significant ($p = 0.004$).

Subsequently, the FEBIM group extended its investigations into the effect of vaccines and showed that vaccination with Bacille Calmette–Guerin (BCG) and vaccinia, but not influenza vaccine, early in life had a similar protective effect as severe naturally occurring infections. The odds ratio, after correction for a number of possible confounding factors, for the risk of melanoma was 0.40 (95% confidence intervals: 0.18–0.85) for recipients BCG, 0.41 (CI: 0.25–0.67) for recipients of both BCG and vaccinia and 0.60 (CI: 0.36–0.99) for recipients of vaccinia alone.[14,15] In a subsequent study, the 17D yellow fever vaccine was likewise shown to confer protection against melanoma.[16]

The protection afforded by infections and vaccinations was neither cumulative nor synergistic, suggesting that they protected by similar mechanisms. In a further study it was found that among those who developed melanoma, a past history of various febrile infections and also BCG and/or vaccinia vaccination resulted in a significantly prolonged survival time, with similar survival for those vaccinated with BCG alone, vaccinia alone and with both vaccines, with a hazard ratio (the relative risk of dying at a given time point) for all vaccines of 0.50 (CI: 0.32–0.78).[17]

These epidemiological findings suggest that the infections and vaccinations conferring protection induce, by cross-reacting immune reactions, clones of T cells that detect melanocytes expressing changes linked to possible future malignant transformation and either repair or destroy them. Most melanomas express a peptide, HERV-K-MEL, on the melanocyte cell surface. The genetic information is encoded by a human endogenous retrovirus of the HERV-K family. Gene products of this virus, in particular the viral envelope protein, are putatively involved in oncogenesis. A patient with melanoma recruited for a clinical trial was found to have a population of cytotoxic T cells recognising the HERV-K-MEL epitope with the amino acid sequence (A)MLAVISCAV.[18] A search of peptide sequences revealed homologous sequences predicted to cross-react with this melanoma epitope in BCG, vaccinia, the 17D yellow fever vaccine and the causative organisms of those infections associated with protection against melanoma.[10,16] This finding supports the hypothesis that infections play a role in establishing protective immune responses to a range of dangers, and it also suggests possible immunoprophylactic and immunotherapeutic strategies. In this context, analogues of the HERV-K-MEL epitope are present in several mycobacterial species in addition to BCG for which peptide sequences are available; namely, *Mycobacterium tuberculosis*, *M. fortuitum*, *M. marinum* and *M. leprae*, suggesting that it is widely distributed in this genus and that a strain other than BCG could, for reasons discussed below, be used as an immunotherapeutic agent.

Other melanoma antigens may also be involved in immune protection and in this context small numbers of $CD8^+$ T cells reactive to Melan-A, an immunodominant antigen present on almost all melanomas have been found in HLA-A2 healthy subjects and are thought to be induced by an exogenous cross-reacting antigen rather than Melan-A itself. In a healthy control subject with no evidence of melanoma, an expanded oligoclonal population of such cross-reactive T cells was found and appeared to have been induced by a mycobacterial protein.[19]

The FEBIM studies point to two different modes of action of anti-melanoma immunity, although they may be closely related. The one mode seems to be an immune repair which is most effective many years before the tumour manifests clinically, as described in Section 5 below. It also appears to influence the susceptibility of melanoma to the effects of the diverse immune reactions against the tumour many years

later. The other mode is principally cytotoxic in nature and the mechanisms, particularly when induced by febrile infections, may include those similar to those mediated by Coley toxins. Obviously both modes depend on the detection of 'danger signals' on those melanocytes which are at risk of becoming malignantly transformed, or which are already transformed, and in appropriate responses to them. Despite these protective mechanisms, various defects prevent their effective operation in those developing the disease.

3. Immune surveillance and immunoregulatory networks

There is increasing evidence that the immune system does not just respond to invasion by 'foreign' agents, notably pathogenic micro-organisms, but conducts an internal surveillance to detect and respond to 'danger'.[20,21] This surveillance depends, in turn, on complex networks of regulatory T cells which are induced by repeated interactions of antigens, both endogenous and exogenous, with antigen-presenting cells, especially the dendritic cells (DC).[22] Self antigens leading to immunological tolerance are derived, at least in part, from cells dying by the process of apoptosis as under normal circumstances presentation of such cells to DCs does not result in their maturation and subsequent activation of effector T cells but to suppression of self-reacting T cell clones.

There is growing evidence that the establishment of beneficial regulatory networks requires a series of infections that millions of years of evolution have led the immature immune system to 'expect' and that isolation of young children from exposure to such repeated infections leads to an increased incidence of conditions resulting from immune dysregulation, including asthma, allergies, autoimmune disease and vasculitis. This concept, known as the hygiene hypothesis,[23,24] has also been advanced to explain the higher incidence of leukaemia in children likewise isolated from sources of common infections.[25]

Related to the hygiene hypothesis is the concept that the pattern of immune reactivity at any given point in time is a consequence of the 'biography' of the immune system[26]; namely, the immunological memory, or 'environmental echoes', of past experience of antigenic challenges.[24] Two particular aspects of this concept are of significance to the outcome of a disease process. First, the sequence of infections affects the ultimate pattern of immune responses, a concept known as 'Original Antigenic Sin', and this may profoundly affect beneficial and detrimental responses in various disease states. In this context, there are several sub-types of memory cells – central and effector – with different functions and which, depending on sequential infections, may vary considerably over time.[27]

Secondly, sequential infections do not just increase the number and diversity of memory cells; instead, as a result of a homeostatic mechanism to limit excessive T cell expansion, a natural infection or immunisation can cause the loss or attrition of memory cells induced by previously encountered heterologous infectious agents.[28] Thus after any infection a new homeostasis of the memory T cell population is established and this could affect protection against tumours. It is possible that repeated infectious challenges could, by reducing memory to heterologous, species-specific, epitopes, focus the immune responses on more widely shared antigens of pathogens.

4. Regulatory T cells – friend or foe?

Although regulatory networks are essential to the correct functioning of the immune system, abnormal or dysregulated networks have harmful effects including induction of autoimmune processes, maintenance of chronic infection and inflammation and to a failure of the immune system to respond efficiently to 'danger signals' expressed during malignant transformation, resulting in the establishment of immune tolerance. Thus the cells responsible for immune regulation, the regulatory T cells (Treg), have been termed 'a dangerous necessity'.[29]

Most Tregs are $CD4^+$ T cells characterised by expression of the markers CD25 and FoxP3 and have an inhibitory effect on other T cells, playing a key role in establishing immunological tolerance to self-antigens. Increased numbers of these cells are found in the peripheral circulation and in the tumour microenvironment in those with advanced and progressing cancer and there is evidence that they inhibit immune control of the tumours.[30,31] Other types of suppressor T cells that may inhibit anti-tumour immune responses include Th3 cells, a class of T cells that secrete the cytokine TGFß and suppress Th1 T cell functions, and a class of natural killer T cells termed invariant NKT (iNKT) cells, though the latter may augment as well as suppress anti-tumour immune responses. The need to consider direct or indirect means of circumventing such suppressor activity in tumour immunotherapy has therefore been stressed.[32]

The specific epitopes recognised by Tregs in immune responses in melanoma, and methods for differentiating between those that enhance or suppress protection, remain to be determined. It is possible that the hygiene-related factors and history of infections and vaccinations described above affect the induction of the various functional types of Tregs.

5. An interplay between repair and cytotoxic mechanisms in melanoma immunology

Immune repair mechanisms are less well characterised than cell-destroying ones in oncology and their possible importance for prevention, and as a basis for successful therapy, is mainly suggested from epidemiologic studies such as those by the FEBIM group,[10,13–15,17] and others.[9] It was suggested that a cell repair mechanism induced by immune recognition of melanocytes in the initial stage of malignant transformation suppresses the expression of the envelope gene of human endogenous retroviruses K (HERV-K) which, if the corresponding env protein is produced, leads to the biosynthesis of a 'melanoma melanin'. A likely repair mechanism involves soluble factors transmitted via cell to cell contacts by $CD8^+$ suppressor T cells. These factors include gangliosides which mediate repair by normalisation of malignant phenotypes as proven in various pre-malignant cell lines, suppression of growth of human melanoma cell lines and reduction

of oxidative stress.[10] This in turn leads to a reduction in tolerance to oxidative stress within the affected cell resulting in successive DNA damage and continuing malignant transformation which the repair mechanism can no longer prevent.

In theory, the difference between such an immune repair mechanism and a cytotoxic one is that the former is not affected by selective pressure leading to subversion of immune recognition and response. The timing of the protection conferred by yellow fever vaccination, around ten years before the clinical presentation of melanoma, suggests that it operates at a very early stage of malignant change and may therefore mediate repair.[16] On the other hand, the significantly enhanced survival rate after surgical treatment of the tumours in patients appropriately vaccinated, as described above, or with a history of relevant infections may indicate that cytotoxic responses against established tumour cells are affected by the same determining factors.

6. Determinants of the qualitative aspects of immune responses

Although it is the epitopes processed by dendritic cells and other antigen-presenting cells (APC) that ultimately determine the specificity of T cells of the various types, they are not the prime determinants of the overall pattern and qualitative nature of the immune response. In recent years the importance of the overall structure of the organism or cell bearing the epitope(s) has become apparent. Thus APCs bear, in their cell membranes, a number of so-called Pattern Recognition Receptors (PRR), of which the most thoroughly studied are the Toll-like receptors (TLRs), of which at least ten are present in humans,[33] that recognise various bacterial and other adjuvants,[34] and thereby conduct a 'mini taxonomic exercise' on the organism.[35]

Activation and maturation of APCs require the interaction of PRRs with the relevant adjuvant molecules of a given micro-organism. The complement of such microbial adjuvants is termed the pathogen-associated molecular pattern (PAMP), although this is a misnomer as not all organisms thus recognised are pathogenic. It is important to note that cancers, having no PAMP, are restricted in their ability to activate APCs especially, as mentioned above, when the antigens are presented as a result of apoptosis.

A number of different PAMP-associated adjuvants have been described and some are under investigation as anti-cancer agents,[36] but it is probable that a combination of such adjuvants activating several PRRs or even the complete PAMP of an infectious agent would exert a more powerful anti-tumour effect.[37] Accordingly, an agent for the immunotherapy of cancer might contain a whole micro-organism bearing a suitable adjuvant complex, or PAMP, that would elicit an effective immune response to epitopes in the agent that elicits clones of T cells and, perhaps, natural killer (NK) cells that cross-react with tumour and cause cytolysis or induce other tumouricidal mechanisms. Other important beneficial effects of such an agent would be to down-regulate, possibly by attrition as discussed above, regulatory T cell populations that, by inducing tolerance, have adverse effects on anti-cancer immunity. In this context, studies in mouse models showed that the addition of PAMP to tumour vaccines in order to induce persistent of Toll-like receptor activation resulted in a bypass of immune tolerance.[38]

In respect to the use of PAMP to activate the innate immune system, the reported beneficial effect in the use of total body irradiation before immune reconstitution in patients with advanced melanoma may, in part, be due to such activation by microbial endotoxins. These exert their effects by translocating across the radiation-damaged intestinal wall and suggest the need to develop ways of inducing the same PAMP-associated effect in a less traumatic manner.[39]

7. How melanoma cells may be killed

The way in which immune responses induce or influence tumour cell death is crucial to the development of immunotherapy, as well as other forms of cancer treatment. Thus, cells may present 'kill me' as well as simply 'danger' signals.[40]

Apart from senescence and catastrophic events occurring during cell division, there are two main ways in which cells, including cancer cells, die or may be killed – necrosis and programmed cell death, of which the latter is divisible into two principal types – Type 1 or apoptosis and Type II or autophagy. The nature of cell death in a tumour has important consequences for the processing of antigen by dendritic cells (DC) and the induction of anti-tumour immune responses. Studies on melanoma have shown that DCs recognise cells dying by apoptosis and by necrosis by means of different cell membrane receptors, with heat shock proteins on the surfaces of necrotic cells being recognised by the CD91 receptor, and that only cells dying of necrosis promote DC maturation. By contrast, uptake by DCs of tissue cells dying of apoptosis may be a mechanism for developing self-tolerance and apoptotic tumour cells may utilise the same mechanism to develop tolerance.[41] On the other hand, it has also been shown, in vitro, that the addition of various cytokines including IL-1 enables DCs that have phagocytosed apoptotic melanoma cells to mature and induce $CD8^+$ T cells specific for certain melanoma epitopes to expand clonally and to kill target tumour cells.[42] In this context, the IL-1 receptor on the DC is structurally and functionally similar to the Toll-like receptors described above.[43] In addition, DCs activated through Toll like receptors 7 or 8 may directly induce apoptosis of tumour cells,[44] showing that DCs have important effector as well as initiatory roles in tumour immunity.

8. Melanoma-associated antigens

In common with all human malignancies, melanomas and also to some extent their putative ancestors, congenital and dysplastic naevi cells, express various tumour-associated antigens that could serve as targets for natural and therapeutically induced immune responses. The principal antigens are listed in Table 1. Tumour-associated carbohydrate antigens (TACA) arise as a result of defective glycosylation. High levels of expression of some of these are characteristic of more aggressive tumours and an increased risk of metastasis.[45] Aberrant glycosylation is more likely to occur in rapidly dividing cells and some TACAs are involved in cell-cell adhesion

Table 1 – The principal groups of antigens detectable on melanoma cells.

- Non-mutated differentiation antigens, including an immunodominant peptide antigen Melan-A (or MART-1/Melan-A).
- Cancer/testis antigens expressed in the testis, embryonic tissues and cancers and include several families including MAGE, NY-ESO-1 and PRAME (preferentially expressed antigen of melanoma) which are found in several cancers but especially melanoma.
- Antigens arising as the result of unique mutations including point mutations in normal genes.
- Over-expressed antigens such as heat-shock proteins.
- Tumour-associated carbohydrate antigens.
- Virus-encoded antigens, including HERV-K-MEL coded by DNA of an endogenous retrovirus.

and extravasation processes which favour establishment of metastases.[46] It is likely that, by loss of the terminal carbohydrate molecules, some TACAs will resemble bacterial antigens and thereby become targets for immune responses induced by bacteria-based immunotherapeutic agents, as discussed below. Virus-encoded antigens include, as described in Section 2 above, those such as HERV-K-MEL which are encoded by an endogenous retrovirus.[10]

Accordingly, there are many potential targets for immune attack of melanomas and several have been used in potential immunotherapeutic agents but it is much more important to develop strategies for overcoming tolerance and other factors that block immune attack.

9. Towards rational immunotherapy for established melanoma

Melanoma has been especially subjected to studies on immunotherapy and a PubMed search in Febuary 2009 with the key words 'melanoma' and 'immunotherapy' yielded over 5000 publications. The considerable diversity of approaches to the development of immunotherapy for melanoma, of greatly varying cost, complexity and burden to the patient have recently been reviewed,[47] with the comment that 'Despite their ability to induce ... occasional clinical responses, cancer vaccines have yet to produce reliable and reproducible clinical tumour regression'.

A number of epitopes expressed on most or all melanomas but not on normal tissue especially the immunodominant antigen, Melan-A, which leads to the generation of clones of highly avid $CD8^+$ cytotoxic T cells,[48] have been used in immunotherapeutic agents of various types. Unfortunately, though, established melanomas have, as described above, a number of mechanisms for evading immune defences, the principal ones being inhibition of the maturation of antigen-presenting cells (due to a lack of PAMP) and induction of immune tolerance. Accordingly, more recent immunotherapeutic approaches to melanoma include agents that overcome tolerance and activate APCs, particularly the dendritic cells. Although single antigens have been used as therapeutic vaccines, target epitopes can be lost as the disease progresses. In one case, recurrence of melanoma was attributed to the loss of expression of the dominant antigen, Melan-A referred to above, due to a failure of its transportation to the cell surface,[49] indicating the need for immunotherapeutic agents based on several cross-reacting epitopes.

In view of the recognised importance of PAMP in the activation and maturation of APCs, attempts have been made to use PAMP-like molecules as immunotherapeutic agents for melanoma. An experimental agent, PF-3512676, activates TLR 9 and in one patient its use led to extensive necrosis of visceral metastases of melanoma.[50] Imiquimod, a synthetic activator of TLR 7, applied topically to accessible metastases of melanoma together with intralesional IL-2 led to local clinical responses as well as to a systemic reversal of the Th2 drift which is characteristic of advanced melanoma.[51,52] Some other PAMPs are being evaluated in Phase I and Phase II clinical trials for various cancers.[36]

While these approaches are of great interest they have, to date, only achieved durable clinical responses in an unpredictable minority of melanoma patients or, in the case of imiquimod with IL-2, are only applicable to accessible lesions. Furthermore, a serious problem, encountered with systemic immunotherapy targeting melanoma differentiation antigens, is the triggering of autoimmune phenomena leading to destruction of melanocytes in the eye, indicating the need to avoid, if possible, the use of cross-reacting epitopes in immunotherapy of melanoma.[53] Activation of TLRs by single agonists may also induce untoward autoimmune phenomena, thus imiquimod which, as mentioned above, activates TLR 7, led to exacerbation of psoriasis,[54] whereas heat-killed *Mycobacterium vaccae*, discussed below, presenting the PAMP of an entire micro-organism, has shown efficacy in the treatment of this condition.[55]

There is no doubt that patients with melanoma develop clones of T cells recognising several antigens on their tumours and yet, owing to various mechanisms of immunosuppression, these appear ineffective in established tumours. Furthermore vaccination with antigens recognised by these T cells leads to effective clinical responses in only a small minority of patients. Notably, in those few patients in whom immunotherapy does succeed in breaking local tolerance, tumour destruction is effected by a wide range of T cells recognising many different antigens including those not present in the immunotherapeutic agent.[56] This phenomenon is illustrated by the induction of a long-lasting clinical remission in a single patient by use of the technically complex technique based on *in vitro* expansion by cloning and infusion of autologous $CD4^+$ T cells specific for the antigen NY-ESO-1 that was present on the patient's melanoma.[57] Not only did this procedure cause the patient to develop immune responses to NY-ESO-1 but also to other melanoma antigens, suggesting a generalised breakage of immune tolerance.

Novel approaches to immunotherapy should therefore focus on the adjuvant-mediated activation of the innate immune system resulting in more effective maturation of

APCs and recruitment of clones of helper and cytotoxic T cells that will lead to the generation of tumouricidal mechanisms and breakage of tolerance by immunomodulatory activity. We postulate that these two properties are present in immunotherapeutic agents that can be readily and economically produced, administered by intradermal injection, or possibly orally, and with minimal or no adverse effects.

10. Mycobacterial products and immunotherapy of cancer

Extensive epidemiological studies showing that cancer and tuberculosis rarely co-exist in the same person[58] led to the attempted use of the tuberculosis vaccine Bacille Calmette–Guérin (BCG) as an immunotherapeutic agent for cancer, notably for melanoma and leukaemia. Although some good responses were noted, the overall results were disappointing and the method was largely abandoned.[59] (Interestingly, though, two studies reporting successful use of BCG cell wall adjuvants for treatment of lung cancer were conducted in Japan, a country where neonatal BCG vaccination is widely used.[60,61])

An exception to the generally disappointing results with BCG is its established intravesical administration for the treatment of superficial bladder cancer in which the BCG would be in contact with, or in close proximity to, the tumour.[62] In this context, intralesional injection of BCG into recurrent cutaneous nodules of melanoma caused regression in 90%, and also in 20% of uninjected nodules in the same lymphatic drainage region, although there was no apparent effect on metastatic disease in deep organs.[63] The effect was thought to be immunologically mediated as the response rate was related to the 'immunocompetence' of the patient.

A major disadvantage of BCG as an immune modifying agent, even as a vaccine against tuberculosis, is that, depending on prior environmental sensitisation, it can boost a range of pre-determined immune responses, both Th1- and Th2-mediated. The advantage of using alternative mycobacterial agents in immunotherapy has been discussed in detail elsewhere.[36,37,64,65] In brief, heat killed preparations of *M. vaccae* prepared in borate-buffered physiological saline at pH 8, have been shown to down-regulate inappropriate immune responses in tuberculosis, leprosy, various allergic and autoimmune conditions including vasculitis. This effect is expressed, though perhaps superficially, as a down-regulation of Th2 responses and an up-regulation of Th1 responses.[66]

On the basis of theoretical considerations and anecdotal observations, a number of clinical studies of the use of *M. vaccae* in the treatment of cancer have been conducted and it was found to significantly prolong survival in inoperable adeno-, but not squamous, carcinoma of the lung,[67] and to be as effective as the more toxic and costly cytokine-based biotherapy in renal cell cancer.[68] In addition, this form of immunotherapy significantly improved the quality of life of patients with lung cancer.[69,70] Although not evaluated in a controlled clinical trial, a study on ten patients with advanced prostate cancer revealed a predominant Th2 cytokine profile before immunotherapy. Levels of PSA declined in five patients, including two who had no therapy other than *M. vaccae*, and in three of these five patients the proportion of peripheral blood mononuclear cells (PBMC) secreting Il-12, indicative of Th1 phenotype, increased.[71] It was concluded that the proportion of PBMC secreting IL-12 might prove to be a potential marker of response to immunotherapy.

Likewise, the effect of *M. vaccae* immunotherapy of melanoma has not been the subject of a formal controlled clinical trial but a preliminary phase I-II study was conducted on patients with advanced stage IV (AJCC) disease.[72] Twenty-four patients received three injections of *M. vaccae* at 15-d intervals, then monthly, and the cytokine profiles of peripheral blood lymphocytes were determined before each injection. Sustained induction of the Th1 cytokine IL-2 was observed within the first three injections, and was maximal at 8–12 weeks in nine of the patients who all showed improved survival in comparison with historical controls and in comparison with those in whom such cytokine induction did not occur. In another small study, three partial remissions were seen in 16 melanoma patients with measurable metastatic disease receiving monthly *M. vaccae* and low-dose IL-2.[73]

11. Summary and conclusions

Melanoma is an aggressive cancer, increasing in incidence in many parts of the world and still carrying a poor prognosis. Despite reports of spontaneous immune-mediated regressions, the many immunotherapeutic strategies, of varying complexity and toxicity, have yielded variable and unpredictable results.

Clinically evident melanomas are the result of an immune-driven evolution ('immunoediting') and are therefore resistant to attack by immune reactions at their current state of regulation which is affected by the 'biography' of the immune system. This in turn is affected by complex environmental factors, including past infections and vaccinations. Protection from immune attack appears to be due principally to mechanisms of tolerance and suppression of programmed cell death. (By analogy, a Th2 driven suppression of programmed cell death may explain the adverse effects of Th2 immune reactivity in tuberculosis which is due to an intracellular pathogen.) In common with tuberculosis, protection of melanoma against attack is associated with immune dysregulation, associated with a 'Th2 drift'.

Epidemiological and clinical observations indicate that certain natural infections and vaccinations (BCG, vaccinia and yellow fever) confer protection against melanoma and reduce the hazard ratio of death in those who develop the disease. Yet as a therapeutic agent for established melanoma BCG has yielded very variable therapeutic results, possibly due to the same factors that affect its very variable protective efficacy against tuberculosis.

Simply vaccinating melanoma patients with tumour-derived epitopes, although these may generate populations of potentially effective $CD8^+$ T cells, is generally ineffective due to the phenomenon of immune tolerance. Strategies to break such tolerance based on the use of adjuvants to establish different patterns of immune responsiveness are therefore required.

Individual adjuvants that activate PRRs on antigen-presenting cells have been studied but there are theoretical and

practical reasons why the adjuvant complement, or PAMP, of a whole micro-organism, also bearing epitopes that cross-react with tumour epitopes, would be preferable.

Epidemiological studies point to the genus *Mycobacterium* as a source for such PAMP/epitope-based therapeutic agents, but as BCG appears to be an immunological 'two edged sword', the use of other mycobacterial species that regularly swing the Th1/Th2 balance towards Th1 would be preferable. The available evidence indicates that certain chromogenic rapidly-growing mycobacteria would be the most suitable, and experimental and clinical studies on one such species, *M. vaccae*, strongly support this conclusion.

Further work is required to determine the mode of action of mycobacterial preparations, particularly the effector mechanisms by which cancer cells are destroyed. If lymphokine-activated killer (LAK) cells are involved, a combination of the mycobacterial preparation and systemic IL-2 might, as suggested by one clinical study referred to above,[72] prove highly effective.

Conflict of interest statement

None declared.

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