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Review Article

Darwinian Factors in the Clinical Expression of Multiple Sclerosis

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Abstract

Many predisposing factors for multiple sclerosis (MS), such as HLA types and geomagnetic fields have been described but the search for a single essential factor has been like searching for the rainbow's end. The most notable feature in the epidemiology of MS in the Western world has been its rise from unknown to the most prevalent disabling neurodegenerative disease of young adults. We suggest that this may be largely or entirely attributable to societal changes that have increasingly isolated populations from micro-organisms that form part of the human microbiome and which are essential for an effective maturation of immune defence mechanisms. This Darwinian explanation suggests a rational approach to both prevention and treatment of MS, by substituting for the loss or absence of factors that millions of years of evolution have led the immune system to 'expect' to encounter early in life.

Keywords: Multiple sclerosis; Darwinian medicine; Microbiome; Neuromelanin; Helminth; Original antigenic sin

Abbreviations

BCG: Bacille Calmette-Guérin; CDMS: Clinically Definite Multiple Sclerosis; CIS: Clinically Isolated Syndrome; EBV: Epstein-Barr virus; HERV: Human Endogenous Retrovirus; HLA: Human Leucocyte Antigen; IL: Interleukin; INF-α: Interferon Alpha; MAIT: Mucosal-associated Invariant T Cells; MAMP: Microbe-associated Molecular Pattern; MHC: Major Histocompatibility Complex; MRI: Magnetic Resonance Imaging; MS: Multiple Sclerosis; TCR: T Cell Antigen Receptor; TNF-α: Tumour Necrosis Factor Alpha; Treg: Regulatory T Cell; PRR: Pattern Recognition Receptor; TLR: Toll-like Receptor; RA: Retinoic Acid

Introduction

Current treatment strategies for multiple sclerosis (MS) are essentially empirical because no single clear underlying cause of the disease has been determined. Numerous genomic and environmental risk factors for MS have been described, but problems of cause and effect remain unresolved. It has indeed been stated, with considerable justification, that MS research is "low on fact, high on fiction" [1].

Although there is a well-described genetically determined predisposition to MS, the association is far from complete as, for example, the risk of an identical twin sibling of an affected person developing MS is only 30% [2]. Thus any genes involved are likely to be of low penetration and influenced by the expression of some or many other genes. Notwithstanding, a large number of genetic loci, including many coding for HLA, have been determined and fine-mapped [3-6]. These studies have demonstrated a central role for the immune system in the aetiopathogenesis of MS but the highly complex nature of the data will require novel tools for their analysis before any unifying factor can be delineated [7].

In this context, it cannot be assumed that studies on genetic predispositions, however detailed, will necessarily provide clues to the underlying cause of a disease process. By analogy, complex genetically determined risk factors have been described in many infectious diseases including tuberculosis and leprosy [8-10] but in each case the underlying cause was not identified by genomic analysis but by isolation of micro-organisms. As described below, certain infectious agents, notably the Epstein-Barr virus (EBV), have been aetiologically associated with MS but the association is not a simple issue of infection [11].

A further potential problem encountered in the search for a single underlying causative factor of MS is that it does not appear to be just one disorder but a cluster of closely related conditions [12]. It is often stated that MS is an inflammatory disorder with an autoimmune basis but it could also be a primary neurodegenerative condition with autoimmunity and inflammation as secondary features [13]. Studies on early lesions in which myelin destruction precedes inflammation suggest the latter and it has therefore been postulated that MS is an 'immunological convolution' involving a primary degenerative condition and an aberrant pattern of host immune reactivity [13]. A claim has also been made that MS is a 'neurocristopathy', implying that it is essentially a developmental disorder of the neural crest [1]. This would explain the higher risk of other neurocristopathies including neurofibromatosis-1, cerebral glioma, glioblastoma multiforme and hypertrophic peripheral neuropathy in MS patients. In this context melanoma has also been defined as a neurocristopathy as it arises from developmentally defective melanocytes of neural crest origin [14] and it has been postulated that many genes expressed during embryogenesis affect the ultimate development of melanoma and may be a more important causative factor than sunlight [15].

We have previously postulated that a melanoma-like neuromelanin may play a crucial role in the aetiology of MS and have suggested how an abnormal iron-enriched melanin could contribute to neuronal damage by failing to appropriately transform reactive oxygen species and oxygen radicals to harmless species but generating

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longer-living reactive species [16]. The involvement of an iron-enriched polymer could explain the claims that the risk of developing MS is higher in regions subject to geomagnetic disturbances [17]. Further studies are required to determine whether (pre-) MS lesions are regularly associated with abnormal types of neuromelanin and, if so, whether they are essential to the development of MS. Irrespective of that, there remain questions of whether there are overriding environmental factors that significantly contribute to explain the changing epidemiological trends in the prevalence of MS.

The changing environment

Epidemiological studies reveal that environmental factors play key roles in determining the prevalence of MS. There is evidence that MS was seldom, if ever, encountered in the industrialised northern nations until the beginning of the 20th century. It is highly improbable that, had MS occurred in the United Kingdom during the Victorian era, eminent observational neurologists such as Hughlings Jackson (1835 – 1911) would not have recognised it and described it. By contrast, in 1930, another eminent British neurologist, Russell Brain (1895-1966), remarked that 'disseminated sclerosis', as MS was then known, was the commonest neurological disease after syphilis in the United Kingdom [18]. An epidemiological study based on World Health Organization mortality data showed that the incidence of MS did indeed rise during the 20th century, reaching a 'veritable MS epidemic' [19].

In the present era, MS is rare in the developing nations and among those born in such regions but migrating later in life to developed nations; conversely, those born in developed nations but subsequently migrating to developing ones retain the risk of the former [20,21]. The second generations have the same risk as the indigenous population, indicating that environmental factors experienced very early in life play central roles in determining whether the disease will develop in a susceptible individual.

A prominent feature of modern day life in the industrially developed nations with, for example, changing practices of animal farming, is the increasing isolation of populations from micro-organisms in the environment, exposure to which is essential for the maturation of the immune system and the development of immunoregulatory networks. The so-called hygiene or 'old friends' hypothesis or Darwinian medicine explains the considerable rise in the prevalence of a class of diseases characterised by chronic inappropriate inflammation [22,23], which may be regarded as a synonym for dysregulated immune reactivity [24]. These disorders include allergies, asthma, vasculitis, autoimmune disease, inflammatory bowel disease, depression, neuroinflammatory and neurodegenerative disorders including MS and some forms of cancer [25] Among the 'old friends' are the helminths which, until recently in human history, were universal inhabitants of the human intestine and vasculature [26-28].

The 'microbiome' and its role in maintenance of health

In the light of the 'old friends' hypothesis, there is currently considerable interest in the natural microbial population of the human body, termed the microbiome. The number of micro-organisms in the microbiome exceeds the number of cells in the human body by ten to one, and the vast majority are on the 400 m^2 surface of the intestinal wall. There are as many as 1,000 different bacterial species in the human microbiome.

As eloquently reviewed by Fleming [26] the intestine is faced with the daunting task of eliminating pathogens while hosting beneficial commensals. The complex immunoregulatory balance required for this task has been termed "anti-pro-inflammatory" [27]. The intestine is a grossly overlooked immune organ, despite the fact that it contains 60% of the body's T cells, 80% of antibody-synthesising plasma cells and the majority of the tissue macrophages (it is also the largest endocrine organ of the body, as well as containing more neurones than the spinal cord). The sub-mucosal layer of the intestine contains enormous aggregates of T and B lymphocytes which in any other tissue or organ would be taken as evidence of an active inflammatory process [26].

It is therefore likely that disturbances in the microbial flora of the intestine initiate a range of disorders characterised by immune dysregulation, including MS. In this context, a case-control study in Denmark showed an association between prior antibiotic usage and the risk of MS (odds ratio 1.41) and it was postulated that the underlying treated infections were causative factors in the disease [29]. Alternatively, by their profound effects on the intestinal flora [30], antibiotics could predispose to MS by disturbing immunoregulatory networks usually maintained by the flora.

While the main immune cells responsible for the immunopathological processes in MS are Th1 and Th17 subsets, regulatory T cells (Tregs) have been identified as potentially beneficial. The suppressor functions of Tregs are impaired in MS [31], and increased numbers of a less suppressive subset of Tregs are found in the peripheral blood of patients with MS [32]. The precise role of Tregs in the pathogenesis of MS remains poorly understood. Reduced numbers of these cells are found in the peripheral blood in patients in remission, compared to healthy controls, but their numbers increase during relapses, suggesting that they do not have a primary role in causing relapse but a secondary one in response to inflammation [33]. On the other hand, functional studies using in vitro suppression assays provide strong evidence of severe impairments in Tregs from MS patients, possibly due to reduced surface expression of inhibitory molecules such as CLTA-4, TIM-3 and TIGIT, poor secretion of immunoregulatory cytokines such as IL-10 and impaired migration of Tregs to the central nervous system [34-37]. Moreover, the intestinal microbiome is able to induce the expansion of a population of Tregs, resulting in suppression of neuro-inflammation in experimental autoimmune encephalomyelitis [38,39].

A further important association between immunoregulatory activity in the intestinal wall and MS is suggested by the recent discovery of a class of lymphocytes termed the mucosal-associated invariant T cells (MAIT) which have a regulatory effect on Th1 immune reactivity in this disease [40]. MAIT are mostly CD161++/CD8+ T cells with the unique Vα19-Jα33 T cell antigen receptor (TCR)-α [41]. They are particularly common in the intestinal *lamina propria* and require an interaction with B cells and intestinal flora for their development and proliferation [42]. Instead of, or in addition to, recognising classical MHC-presented antigens, the MAIT TCR binds to vitamin B metabolites presented by MR1, a MHC class 1-related molecule, enabling them to recognise micro-organisms by their metabolic signatures [43]. Little is known of the functions of MAIT in

human disease but they are present in MS lesions and appear to have a disease-suppressing role; their numbers in the peripheral blood are significantly lower in MS patients than in healthy controls with numbers being reduced even further in those with active disease but with an increase in numbers in those entering remission [40]. It was therefore suggested that the MS-immunosuppressive properties of MAIT could be enhanced by therapeutic modulation of the patients' intestinal microbiomes.

In the context of the above, it is noteworthy that the introduction of the invariant Vα19-Jα33 MAIT gene into non-obese diabetic mice delays the onset of diabetes, another condition characterised by immune dysregulation and autoimmune phenomena [44].

Studies on the influence of the human (and animal) microbiome and its effect on the integrity of the immune system have given rise to the concept of 'commensal-mediated immunomodulation' and the possibility of 'commensal-based therapy' and the relevance to MS has been reviewed in detail [39,45].

'Original antigenic sin'

A further important consequence of the 'old friends' hypothesis is the shift in the timing of certain infections in the 'hygienically advanced' regions of the world with some that in former times were regularly acquired in infancy now occurring years or decades later. In the case of early infections immune responses are directed to dominant antigens of the pathogen while in infections occurring later in life prior infections by other agents bearing cross-reactive epitopes may direct the immune response to other, non-dominant, epitopes resulting in quite different host responses, a concept termed 'Original Antigenic Sin' [46,47]. This concept has been used, for example, to explain why second infections by the dengue virus, of a different serotype, may cause much more serious disease than the first infection [48].

Based on such considerations and our own investigation on a range of environmental factors in a group of German patients with onset of MS in childhood [49,50], we have attempted to develop a unifying concept for the aetiopathogenesis of MS [51-54]. Factors with the most significant associations with MS (after Bonferroni correction) point to a compromised immune reactivity associated with a complex infectious background. In brief, MS appears to be the result of the cumulative effects of many factors which become involved after an innate immune response protecting against MS is compromised by a viral infection, for example, with the Epstein-Barr Virus (EBV) occurring at a point in time when the pattern of immune responses against this virus has already been determined by prior infections. This could result in the generation of different sets of regulatory and effector T cells with possible harmful rather than protective effects. Likely candidate epitopes of Tregs, competing T-helper cells and T-effector cells involved in the immunopathogenesis of MS have been delineated [51].

Endogenous retroviruses

An attrition of MS-protective immune reactivity results in an increased expression of capsid and envelope proteins of human endogenous retroviruses (HERVs) of the H/F, W and K families [52-54]. Being abnormally expressed endogenous antigens, HERV proteins could in principle initiate autoimmune phenomena [55]. It is of note that EBV and other herpes viruses which, as described above, have been linked to the pathogenesis of MS, are able to transactivate HERVs leading to presentation of superantigen [56], indicating that the viral as well as the bacterial composition of the microbiome must be considered.

The role of HERVs in the pathogenesis of MS requires clarification as it is uncertain whether they are initiators or promoters of the disease or merely innocent bystanders [57]. Studies in Scandinavia point to a role for HERV-Fc1 in the development of active disease [58,59] and there is anecdotal evidence that antiretroviral therapy has beneficial effects on MS. A clinical trial on the anti-HIV agent raltegravir (NCT01767701) [60] is in progress and a phase I study of a monoclonal antibody,GNbAC1, against the envelope of the HERV-W expressed in MS (NCT01699555) has been completed, with favourable safety and pharmacokinetic profiles encouraging a phase II trial [61]. These are the first example of trials of the anti-microbial therapy of a viral agent arising endogenously from the human genome rather than from an exogenous source.

A further key element in the pathogenesis of MS is the accumulation of reactive oxygen species (ROS) that might play a central role in this and many neurodegenerative disorders [62]. The mechanism of this accumulation is obscure but, as described above, we have postulated that it is of importance in connection with the formation and oxidative charging of a hypothetically altered neuromelanin [16].

Correcting immune dysregulation

The above concepts may pave the way to the delineation of a single factor underpinning the complex immunopathology of MS and, in turn, a definitive single therapeutic approach. Nevertheless, irrespective of its underlining cause(s) and manifold clinical features, MS appears essentially to be a disorder of immune regulation [13]. If this is the case, therapy should address that which at present is the predominant recognised feature of the disease process; namely, the 'immunological convolution' distinguished by dysregulated patterns of immune reactivity underpinning the chronic inflammation at the core of the disease process.

Disease-modifying drugs are available for the treatment of relapsing-remitting MS, but they all reduce inflammation by targeting peripheral aspects of the dysregulated immune reactivity [12]. A major challenge is to develop an agent that exerts its effect at a much more fundamental level of immune dysregulation and to consider whether any currently available agents developed for other conditions are of actual or potential value.

Two quite different microbial agents have recently been reported to have beneficial effects in MS; namely, mycobacteria, specifically the Bacille Calmette-Guérin (BCG) vaccine, a living attenuated derivative of the bovine tubercle bacillus, *Mycobacterium bovis*, and helminths. These agents, in common with all other microbes, have characteristic arrays of adjuvant molecules comprising the microbe-associated molecular pattern (MAMP) which interact with pattern recognition receptors (PRR) on or in host cells [63]. Around 40 PRRs are known; the most thoroughly studied being the Toll-like receptors (TLR). The MAMP-PRR interactions determine the qualitative nature of the subsequent innate and adaptive immune responses, with the

potential to down-regulate harmful patterns of immune reactivity and induce or enhance beneficial ones. In this context, it is important to note that the immune system does not 'see' adjuvant molecules in isolation but does what has been termed 'a mini taxonomic exercise' on the totality of the presented MAMP [64]. Accordingly the careful selection of a micro-organism bearing appropriate MAMPs could provide powerful immune modulating agents for the treatment of a given disease or group of diseases, as well as playing a preventive role.

Bacille Calmette-Guérin (BCG)

Clinically definite MS (CDMS) is often preceded by an initial transient demyelinating episode termed the clinically isolated syndrome (CIS), which progresses to CDMS in about half the cases within two years [65]. There is firm evidence that vaccination with BCG after an episode of CIS significantly reduces the risk of developing CDMS and the development of gadolinium-enhancing lesions on MRI [66]. The same authors had previously demonstrated that BCG vaccination of patients with relapsing-remitting MS suppresses the evolution of active lesions seen on MRI [67,68], and they speculate that BCG may be acting as an immune modulating agent, correcting immune dysregulation caused by the lack of exposure to 'old friends'. It remains to be determined whether the observed beneficial effect of BCG vaccination in MS is due to the expansion of the population of immunoregulatory cells such as Tregs and MAIT cells, particularly those induced by prior exposure to environmental mycobacteria. An alternative possibility is that, if HERVs are involved in the pathogenesis of MS, BCG vaccination may induce immune responses to HERV epitopes expressed or immune presented on cell surfaces. In this context, BCG vaccination affords a significant degree of protection against melanoma [69], which as discussed above, may have developmental characteristics in common with MS, and a mechanism based on induced immune responses to an expressed and immune presented HERV epitope (HERV-K-MEL) has been postulated [70,71].

Another example of an immune modulating effect of BCG is the demonstration of its beneficial effect in type 1 diabetes [72]. Unfortunately these beneficial effects were short lived and adverse local effects prevent repeated treatment with BCG. In a more recent preliminary safety study six patients with type 1 diabetes received two injections of low doses of BCG [73], resulting in the release of insulin-autoreactive T cells into the peripheral circulation with the majority being dead, thought to have been killed by BCG-induced TNF-α, an increase in Tregs and a temporary increase in fasting insulin secretion.

By coincidence, one of the control diabetic patients developed an acute EBV infection with flu-like symptoms 3-4 weeks after receiving placebo injections and was found to have identical immune responses and an increase in fasting insulin secretion as the BCG-treated subjects. This may further illustrate the possible wide-ranging impact of an EBV infection on effector and regulatory T cell populations which, depending on the 'biography' of the immune system, can be beneficial or deleterious. The conclusion of this study was that higher doses or repeated administration of smaller doses, of BCG would be required although as discussed below, other mycobacterial preparations may well have similar beneficial effects without the complicating hypersensitivity phenomena and other untoward reactions. In this context, *M. bovis*, of which BCG is an attenuated derivative, is just one of well over 150 known species of Mycobacteria, the great majority of which are harmless saprophytes widely distributed in nature, particularly watery environments [74].

The role of BCG vaccination in the prevention of MS has not been investigated although a study in Denmark revealed that the subsequent risk of MS was inversely associated with a positive tuberculin skin test at the age of seven years [74]. It is also noteworthy that, as mentioned above, BCG vaccination early in life affords a useful degree of protection against melanoma [75], which may share some developmental characteristics with MS.

Helminths

In common with Mycobacteria, most helminths are free-living but a few species have evolved to a parasitic existence. It has been shown that the incidence of MS is low in regions in which intestinal infestation with the whipworm (*Trichuris trichiura*) is common and that parasite-infected MS patients experience significantly fewer relapses, better disability scores and lower activity in the lesions on MRI than uninfected MS patients [76]. In a phase I study, five MS patients received pharmaceutical grade, non-pathogenic, living *Trichuris suis* ova every two weeks for three months, resulting in a 70% decline in the number of new gadolinium-enhancing lesions on MRI although the number rose to the pre-treatment level two months after cessation of therapy [77]. A phase 2 clinical trial (NCT01413243) based on 50 patients has commenced.

Towards novel therapeutic approaches

Neither BCG nor helminths are ideal for clinical use. Viable helminths, even if usually not pathogenic, could cause disease in susceptible patients and there is a theoretical risk that they could have the paradoxical effect of enhancing immune dysregulation. [78]. Although being attenuated, BCG, being viable, is also capable of causing disease which may become widespread and disseminated notably in immunocompromised patients and it may cause hypersensitivity reactions in tuberculin positive persons, with repeated dosing amplifying the hypersensitivity. These problems could in principle be overcome by using non-viable preparations or sub-cellular fractions, if they are found to have any effect.

As mentioned above, the immune system does not 'see' individual adjuvants singly but the totality of the presented MAMP. In this context it has been demonstrated that helminths and soluble extracts of helminths predominantly drive TLR-2 immune responses [79]. The expression of TLR-2 (but not TLR-4, -5 and -9) on B cells and dendritic cells from helminth-infected MS patients was much greater than on those from uninfected patients [79].

In a study of the role of TLR-2 in protective immunity in MS, it was demonstrated that serum levels of retinoic acid (RA) were significantly higher in helminth-infected MS patients than in healthy controls and MS patients uninfected by, or treated for, helminths [80]. Signalling via TLR-2 cause's expression of genes (*Adh1 and Raldh2*) involved in RA synthesis in dendritic cells and enables these cells to induce Tregs which suppress the production of proinflammatory cytokines including IL-6, IL-12, IL-23 and TNF-α.

Retinoic acid is an active metabolite of retinol (vitamin A) and an inverse relationship between serum retinol levels and MS lesional

activity on MRI has been described [81]. The pathways through which retinol and RA mediate immune modulation are complex and not fully elucidated although they have been shown to promote the expression of FoxP3, a marker associated with many Tregs [82]. In the case of helminths, they suppress inflammation thereby allowing the parasites to survive for long periods while reducing inflammation and causing minimum tissue damage, a desirable occurrence in MS [80].

Unlike helminths, Mycobacteria are not long-term commensal residents of the human intestine but the numerous non-pathogenic species occur in large numbers in free water and moist natural environments such as marshes [67] and in biofilms lining water pipes. Although they do not appear to replicate in the human intestine they gain regular though temporary access through consumption of water, and have accordingly been termed 'pseudocommensals' [22]. Their numbers are, however, reduced by water treatment and are rarely encountered in bottled waters from deep wells which are increasingly (though unnecessarily) consumed instead of tap water by the general population in the industrially developed nations.

As mycobacteria have the most elaborate and lipid-rich cell walls in all of nature and have genes involved in the biosynthetic pathways of every known class of lipid [83] it is no surprise that they have an enormous range of adjuvant properties and have been incorporated in Freund's adjuvant to enhance antibody production and other immune responses in experimental systems. Although the primary use of BCG is for vaccination against tuberculosis, it has been shown to have far-reaching effects on human health, including the potentiation of the efficacy of other, unrelated, vaccines. For example, BCG vaccination resulted in a 45% reduction in infant mortality due to several causes in Guinea-Bissau and Benin [84], a 50% reduction in death from pneumonia in Brazil and a significant decline in death from malaria in Guinea-Bissau [85]. Studies on the instillation of BCG into the murine bladder (as an experimental model for treatment of superficial bladder cancer) likewise show that it induces expression of a wide range of genes involved in specific immune responses and non-specific inflammation in a number of complex networks [86]. Interestingly, it was also demonstrated that BCG induced the expression of gene products involved in axonal guidance, indicating that immune modulators may have an effect on the nervous system, raising the possibility that they might mediate the repair of MS plaques.

It should be noted that BCG does not induce a single pattern of adjuvant activity. It induces, for example, both Type 1 and Type 2 patterns of immune reactivity with the predominance varying over time following vaccination [87] and with the pattern varying according to geographical location [88]. Thus, for example, infants vaccinated in the UK principally develop Type 1 responses while those in Malawi develop Type 2 responses, possibly explaining the marked geographical differences in the protective efficacy of BCG [89]. Studies in a murine model indicate that exposure to environmental mycobacteria influences immune responses to BCG by inducing populations of Tregs [90].

Alternative adjuvants

The above considerations indicate that mycobacteria and helminths have complex immune modulating activities which vary according to context. One problem is how, on the basis of immunological considerations, to select micro-organisms for therapeutic use. Much emphasis has, in the past, been placed on a 'Th1-Th2 balance' but this now appears too simple a concept. For example, helminths are potent Th2 adjuvants, yet they not only suppress Th1/Th17 mediated inflammation but also Th2-associated allergic reactions [91], most likely due to the induction of Tregs.

As mentioned above, the natural environment contains over 150 species of mycobacteria and contact with them, principally by drinking water containing them, is a regular event. Studies in a region of Uganda where BCG provided a high level of protection against tuberculosis and leprosy led to the conclusion that an environmental mycobacterium, *M. vaccae*, originally isolated from cow faeces from Austria [92], modulated the pattern of immune responses in that region to one that afforded protection against pathogenic mycobacteria and one that BCG vaccination could enhance [93].

Heat-killed *M. vaccae* has been shown in a murine model to enhance Type 1-mediated immune reactivity and to down-regulate Type 2 activity and, perhaps more importantly, to modulate the activity of Tregs [94,95]. Similar impacts of intradermally-administered *M. vaccae* on Type 1 and Type 2 immune reactivity in human beings has been demonstrated, and oral administration was shown to have very similar effects on immune reactivity as intradermal injections, although this was not a direct side-to-side comparative study [96].

There is also evidence from a murine model that heat-killed *M. vaccae* down-regulates allergen-mediated hypersensitivity reactions, although small clinical trials in human asthma and atopic dermatitis yielded less impressive results. The murine studies indicated that immune dysregulation leading to atopy and allergy, and the impact of *M. vaccae,* was at the level of Tregs [96,97] and this is supported by the finding that genetic polymorphisms in FoxP3+ Tregs are associated with the risk of a range of allergic and autoimmune disorders [98]. Furthermore, psoriasis is mediated by inappropriate Type 1 activity and therapeutic attempts are to swing the immune responses to Th2-mediated protective ones are being made [99], yet intradermal injections of heat-killed *M. vaccae* lead to improvements in this condition [100]. A possible explanation of these paradoxes is that Th1 and Th2 cells both have normal and essential functions but in states of immune dysregulation both may function abnormally and cause disease.

The emerging picture is that while tissue-damaging immune reactions are mediated by such different T cells as Th1, Th2 and Th17, the immune defect lies at the level of the Tregs of which there are several functional types [101]. Accordingly more emphasis may well need to be placed on regulatory cells than on the final effector Th1, Th2 or Th17 cells, attempts made to correct anomalies at the level of Tregs by the use of appropriate immune modulating agents, irrespective of the nature of the effector cells. In this context, while in some experimental and clinical situations *M. vaccae* enhances Type 1- and down-regulates Type 2 immune reactivity, it exerts its principal effect on Tregs rather than on Th1 cells directly, in contrast to the bacteria *Acinetobacter lwoffii* and *Lactobacillus lactis* G121 which also reduce allergic reactions in mice but by having a more direct effect on Th1 cells [102]. Accordingly, there appear to be several 'layers' in the process of immune regulation, with some agents acting at a basic level

and others at a more superficial one, a factor requiring consideration in the selection of immune modulators for various clinical purposes. As *M. vaccae* has been shown to be of clinical benefit in disorders resulting from either Type 1 or Type 2 dysregulation it appears to act at a more basic level.

In the light of the above considerations, particularly the shared ability of mycobacterial preparations and helminths to down-regulate untoward immune phenomena facilitating the clinical manifestation of MS, and the demonstrated beneficial effect of BCG vaccination in prodromal and relapsing-remitting MS, the use of a carefully selected mycobacterium as a food supplement would appear a logical step forward.

Conclusion

Emerging ways for preventing MS by vaccination

Vaccination strategies provide the standard means of preventing disease due to specific infections but interest is growing in their use to prevent disease due to immune dysregulation, including depression [103]. In the case of MS, a vaccine could substitute for Epstein-Barr virus (EBV) infection in early childhood which, due to improved standards of hygiene in the industrialized nations, has largely been delayed from infancy to early adulthood [51].

Development of such a vaccine will not be straightforward as the viral EBNA1 protein, postulated by us as being essential for protection against MS [49], and may also be involved in tumour development and progression. Until such a vaccine is developed, trials on the use of helminth derived epitopes and BCG vaccine or other mycobacterial preparations seem very worthwhile.

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