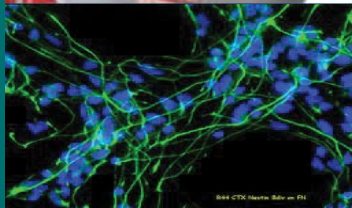
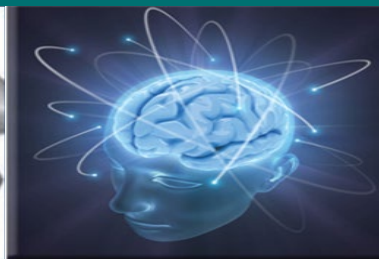


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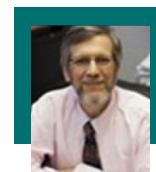
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Perspective

What Could Be a Primary Cause of Multiple Sclerosis: Is It an Autoimmunity Triggered by Chronic Protozoan Infection?

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Abstract The generally accepted paradigm of multiple sclerosis is the autoimmune one; still, a body of evidence suggests that this disease may actually be triggered by an infectious factor. In this paper, it is hypothesized that multiple sclerosis may actually be a rare complication of a protozoan infection, which is usually asymptomatic but in some susceptible individuals is accompanied by autoimmune attack against the nervous tissue. If multiple sclerosis were actually caused by such an infection, then a microorganism responsible should exhibit several properties: it (i) is transmitted by an arthropod vector; (ii) is characterized by specific metabolism of the lipids; (iii) should be dependent on iron; (iv) should be associated with an autoimmune response of the host; and (v) should be susceptible to pharmaceutical agents used for the treatment of multiple sclerosis but not to the degree that would allow its eradication. A combination of these properties suggests a role of a blood-residing protozoan.

Keywords epidemiology; multiple sclerosis; protozoan infections

1. Introduction

For the time being, multiple sclerosis (MS)—a chronic disease of the central nervous system—is a pathology of unknown background. The generally accepted paradigm of MS is the autoimmune one, which means that the disease is caused by autoimmune attack against nervous tissue primarily due to myelin-reactive T cells. It is suspected that a combination of genetic and environmental factors may be responsible. However, it remains unclear how such an autoimmune reaction could be initiated. Also, a number of uncertainties cast doubt on traditional autoimmune models of MS. Although the relative efficacy of immunomodulatory drugs used for the treatment of MS supports the idea that the immune system plays a significant role in the pathophysiology of the disease, several findings from human studies do not fit into the autoimmune paradigm. These experiments suggest that inflammatory processes responsible for formation of MS plaques are not necessarily primarily autoimmune [12, 15, 17, 18, 28, 33, 39, 42, 59, 87, 99, 100, 104, 121, 122].

2. Is MS actually caused by an infectious factor?

A number of papers suggest that MS may actually be triggered by an infectious factor [53, 81, 109]. Yet, in spite of several decades of intensive research, no definitive evidence has emerged to identify a single microorganism as a cause of the disease. In this review, I will examine which microorganism might theoretically be responsible. I will demonstrate that it is likely that MS is a rare complication of a protozoan infection, which is usually asymptomatic but in some susceptible individuals is accompanied by an autoimmune reaction against nervous tissue antigens. I will examine such a hypothetical scenario, first summarizing the results of epidemiological studies that suggest a role of infection in the pathogenesis of MS. Then, the findings of histological and immunological studies on MS will be discussed, followed by a review of pharmacological trials that support a potential role for a protozoan. Finally, I will focus on diagnostic strategies that may be successful in identifying such a microbe.

3. Evidence coming from epidemiological studies: an environmental and not genetic background of MS

The relatively low concordance rate of identical twins indicates a contribution of nongenetic factors to MS etiology [72, 81]. Currently, it is also well known that MS is characterized by uneven geographic distribution. It is widely accepted that people living at higher geographic latitudes, with reduced solar exposure, and consequently manifesting vitamin D deficiency are at higher risk of developing MS. However, even if it is taught that the risk of MS is related to the geographic latitude, with a higher risk in countries located far away from the equator and a lower risk in the tropics, the actual geographic distribution of the disease is a bit different [50]. Although the distribution of MS is following the latitude paradigm, the high-risk

areas comprise northern Europe, the northern United States, Canada, eastern Siberia, southeastern Australia, and New Zealand; while countries located close to the equator are very rarely affected by MS [81,94]. Similarly located at high latitudes, are China, Japan, northern Scandinavia, central Siberia, and Alaska, which belong to low- or moderate-risk areas [94]. The Mediterranean region and Middle East have long been considered low-/moderate- risk areas, but recent studies indicate that these regions are at moderate/high risk [52,94]. A similar situation, with increasing risk for MS, is seen in the southern states of the United States, South Africa, and eastern Australia [4]. In the beginning of the 20th century, MS was primarily affecting Caucasians. The majority of MS patients was living in rural areas, and the female:male ratio was close to 1 [3,81]. Nowadays, the disease is found mostly in women (female:male ratio ranging from 2 to 5), while racial and urban/rural differences are less pronounced [4,116]. Since these changes occurred within a few (sometimes a single) generations, it is rather unlikely that genetically driven factors can primarily be responsible. Still, if MS were caused by a microorganism, a possibility that such a microbe has changed genetically within a century cannot be excluded.

Other observations also cast doubt on the genetic source of MS. These studies examined the fate of people migrating from high- to low- and from low- to high-risk areas. It has been revealed that adult people migrating from Europe (a high-risk area) to South Africa, Australia, and Israel (low-risk areas) retained the high risk for MS of their motherlands. However, those migrating as children (a borderline age was 15 years) presented with a risk similar to the native population of the new country [24,27,52,53,73]. Also, people migrating from continental Turkey (a low-risk area) to Cyprus (a high-risk area) were still at low risk of developing MS [25]. A similar situation was seen in the case of adults migrating from the Indian subcontinent, sub-Saharan Africa, and the Caribbean region (low-risk areas) to the United Kingdom (a high-risk area). The immigrants did not exhibit a high prevalence of MS. Still, while the adult immigrants to the United Kingdom infrequently developed MS, their UK-born children (obviously of the same genetic profile as their parents) presented with the same high risk of MS as native people of Britain [32]. Similarly, although MS is nearly unknown in black Africans [26], it is quite common in black Americans [50,52]. In recent study on US soldiers, the risk of MS was even higher in blacks than in whites, contrary to the widely accepted genetic paradigm [116]. Of note, there are several studies that have demonstrated that the susceptibility to MS was associated with genes of the major histocompatibility complex (MHC) [41,62]. Still, this effect was not very strong. On the contrary, the aforementioned epidemiological observations indicate that genetic susceptibility plays a minor role in MS

pathogenesis and point toward an environmental factor that is responsible.

A number of epidemiological studies have shown that in several areas, the probable epidemics of MS took place. The best known and best documented example of such an epidemic comes from the Faroe Islands [52,53,117]. Importantly, medical records in the Faroe Islands—a north Atlantic territory of Denmark—were scrupulously carried out throughout the 20th century. Then the medical documentation of all Faroese was meticulously reviewed by MS researchers in a search of any anomalous epidemiological trend, thus minimizing a potential bias resulting from inadequate methodology or incomplete data. It appears that the Faroe Islands were free of MS until the islands were occupied by British troops during World War II. A few years after the arrival of British soldiers, the first cases of MS ever found among native Faroese were described. This first outbreak of MS was followed by two possible secondary epidemics [52,53]. Similar epidemics of MS—yet, not so well documented—occurred in other relatively isolated areas: Iceland [53], the Orkneys [53,70], and Sardinia [95]. Well-documented clusters of MS in some areas were also reported [31]. Besides, it seems that there is a spread of MS that affects new areas and new groups of humans (women, nonwhites, older people, urban populations) [4]. Although the prevalence of MS is growing in almost all countries studied, there are some areas (e.g., the Orkneys) [53] where the prevalence is falling, maybe due to an increased resistance of the population to an infectious agent.

4. MS as an epidemic

The epidemic of the disease does not necessarily mean that the pathology is caused by an infectious factor. Quite the contrary, a lot of epidemics are caused by nonbiological factors (tobacco, toxins, air pollutants, radiation, etc). Some researchers claim a role of environmental factors associated with the so-called western lifestyle (western diet, physical inactivity, inadequate sleep, chronic psychological stress, smoking, pollution, etc.). But if it were the case, then a high prevalence of MS should be expected not only in Europe and North America but also in Japan. Besides, MS should not have been known before the 20th century, which is not the case. (Actually, the first description of probable MS comes from the 15th century.) Considering the fact that MS is affecting very different environments (urban and rural areas, highly polluted and pristine territories, countries with high and low levels of background radiation, etc), it is very unlikely that MS is caused by nonbiological factors. Some epidemiological studies point toward a microorganism as being the cause of MS epidemics [31,51,52,53,70,95,117]. Still, it is also known that despite many efforts, not a single microorganism has been definitely identified. If this were a simple issue, however, someone would have already

solved the problem. Perhaps the biology of MS may give us more information about this elusive microbe. Although MS is the most common neurodegenerative disease in young adults in western countries, it is nonetheless a rare pathology, with the prevalence in high-risk areas, ranging from 100 to 200 per 100,000 and being much less common in the rest of the world. Importantly, epidemiological models tell us that if an infection is symptomatic in the majority of infected individuals (like influenza virus) and the prevalence is low, it is impossible that such an epidemic is active for a longer time with such a low prevalence. It is due to the fact that the number of infected individuals in a population is too small to enable effective transmission of the pathogen. Still, some epidemics with such a low prevalence of symptomatic patients may persist, but an alternative reservoir of microorganisms is required, either in humans (in this scenario, the disease is clinically overt in a minority of infected people) or in animals. Sometimes a reservoir of infectious agent may exist both in nonsymptomatic humans and animals. Probably, the hypothetical infectious agent of MS had to be residing initially in animals. All, except for only a few, human infectious diseases are caused by microorganisms that originally were residing in animals, primarily domesticated ones, such as pigs, cattle, and poultry. Since the geographic distribution of MS is uneven, with the lowest prevalence in Africa, it points against microorganisms that have been infecting humans for millennia. Otherwise, MS would be distributed differently, either with the highest prevalence in Africa (the motherland of the mankind) or with the same prevalence worldwide. MS is also uncommon in the main centers of animal domestication: the Middle East (until the recent increase in prevalence), India (except for some non-Indian ethnic groups), and China [94]. It should also be mentioned that in the past, a canine source of MS was considered (canine distemper virus); but this possibility has been ruled out by epidemiological and virological research [6]. In conclusion, this elusive microbe is unlikely to come from typical domestic animals.

5. MS and historical data

Historical records of possible MS cases suggest that the disease is quite new. Contrary to other diseases, not a single description of possible MS can be found in ancient and early medieval texts (respecting the fact that the first medical description of MS as an entity actually comes from the second part of the 19th century) [16]. The first known person with possible MS was St. Lidwina, a Dutch woman who got sick in 1385 [74]. Some researchers claim that a primary focus of an MS epidemic might be situated in the Scandinavian peninsula [51, 52, 56] (far away from the main centers of animal domestication). If MS outbreaks actually began in Scandinavia, it is tempting to suggest that such

an event took place around the 13th to 14th century. It is rather unlikely that it occurred much earlier, considering the geographic distribution of MS and a lack of ancient and early medieval descriptions of possible cases of the disease (even respecting the scarcity of old medical records). Moreover, a suggested chronology is in line with the nonexistence of MS in the Faroe Islands before the 1940s. These islands were settled by the Norsemen (the Vikings) in the early Middle Ages; and from that time (until World War II), the Faroese population, except for sporadic contact, was largely isolated. In general, the Faroese are probably the descendants of those Norsemen who had no contact with a hypothetical infectious agent of MS (interestingly, the only Faroese who developed MS before 1940 were those living for several years in mainland Denmark) [8]. There is also another fact pointing toward the 14th century. One may ask, what has happened in 14th-century Scandinavia that made the jump of a hypothetical infectious agent from an animal reservoir to humans possible? Indeed, the 14th century was somewhat unique. First, at that time, a dramatic climate cooling, the so-called Little Ice Age, took place. It resulted in the migration of nomadic tribes of northern Scandinavia, the Sami people (the Lapps), southward and their intensified contact with Norsemen. Second, at the same time, the Danish, Swedish, and Russian states became stronger and more centralized. Consequently, taxing the people, including the nomads, intensified, again resulting in more contact between the Lapps and Norsemen. Beginning from the 14th century, the Sami people, due to climate change and the necessity of paying taxes, intensified reindeer herding. Reindeer husbandry began in central Norway in the 11th century, but until the 14th century, reindeer hunting prevailed. The question of whether an infectious agent of MS has jumped from domesticated reindeer or whether another arctic animal has played a role will remain, of course, unanswered. Still, it is tempting to speculate that MS originally developed in the Sami people, perhaps long before the 14th century. It is known that the prevalence of MS among the Lapps is much lower than that of their Swedish and Norwegian neighbors [94] (despite the fact that they live in the northernmost part of Scandinavia, thus contrary to the latitude model of MS). But if an infectious agent of MS has been circulating in this population for a longer time, one may expect a decreased prevalence of the disease due to increased resistance and/or less intensive immune responses associated with the infection.

6. Other epidemiological data

In addition to the already-presented epidemiological data, there are also some other data that can help us to identify a hypothetical factor of MS. Positive correlations have been found between MS prevalence and the bovine population, as well as with milk consumption. Although

these phenomena could be interpreted differently, perhaps a link between MS and cows exists [67,68,81]. In a recent epidemiological study on US soldiers, the authors revealed an unexpectedly higher prevalence of MS in the Army and Air Force and significantly lower prevalence in the Navy and Marine Corps [116]. The researchers were not able to find a reasonable explanation for this fact. Was service on the sea somewhat protective? It is tempting to speculate that if a hypothetical microorganism responsible for MS were transmissible by an arthropod vector (for example, mosquito), one should expect to find such a phenomenon, since those militaries serving on the sea were less likely to meet blood-sucking arthropods.

There are also studies revealing obesity in adolescents as a risk factor of developing MS [80]. Although this association of increased body mass index with a higher susceptibility to MS is unclear, and although the possibility that obesity promotes MS through increased inflammation associated with a higher production of adipokines and proinflammatory cytokines cannot be ruled out, perhaps metabolism of lipids plays a role. Such a link is not very likely in the case of bacterial or viral infections. Still, it may be considered if protozoa were responsible. It is well known that many parasitic protozoa (including *Plasmodium*, *Trypanosoma*, and *Trichomonas*) cannot synthesize lipids and have to use these fatty compounds produced by the host [21,35,36,71,86]. Besides, some parasites (e.g., *Trypanosoma cruzi*) are using adipocytes as a safe reservoir, where these microbes can survive and evade the immune responses of humans [34, 82]. Interestingly, a low-fat diet exhibits protective effects against MS progression, as has been demonstrated in some longitudinal clinical studies [106,107,118].

7. Histology of MS plaques and immune responses in the setting of the disease

MS plaques are characterized by profound heterogeneity of the lesions. Several types of these foci of demyelination can be distinguished. Still, all these types primarily present with CD8⁺ lymphocyte and macrophage infiltration [10, 58,102], which is not typical in classic autoimmune disorders, characterized predominantly by CD4⁺ lymphocytic infiltrates. Deposition of immunoglobulins and complement antigens, suggesting an important role for autoimmunity, can only be seen in class II lesions; while in class I and III lesions, a destructive process is probably induced by another undetermined mechanism [64,69]. Immunological studies have also revealed that MS-associated autoantibodies are produced by nervous tissue- and cerebrospinal fluid-residing B cells. Interestingly, these cells comprise a limited number of clonotypes and are not found in the peripheral blood. This antibody response is stable over a long time [42]. Other studies have demonstrated clonally expanded CD8⁺ lymphocytes and persistence of these cells

in the cerebrospinal fluid over a period of months [102]. Such an immune response, in addition to MS, is typical for a variety of acute and chronic infections of the central nervous system. CD8⁺ lymphocytic infiltration may suggest a protozoan infection (such a scenario has already been discussed by Murrell et al., who called attention to similarities between histological characteristics of MS and African trypanosomiasis; still, the authors found such a scenario impossible, considering the geographic distribution of African trypanosomes) [81]. Yet trypanosomes, as well as other parasitic kinetoplastids and apicomplexans, are actually also common in temperate and subarctic climate zones. Of note, parasitic trypanosomes induce the production of autoantibodies against myelin basic protein and galactocerebrosides [2,9,43,88]—a known hallmark of MS [75,84,89]. In general, the histological and immunological picture of MS is neither stereotypic of an autoimmune disease nor representative of a typical bacterial or viral infection. It rather suggests an atypical viral infection or a disease triggered by parasitic protozoa or spirochetes [81].

The histology of early MS plaques may also be helpful. Newly forming plaques are not infiltrated by lymphocytes. Such an infiltrate is typical for viral encephalitides and experimental autoimmune encephalomyelitis (the animal model disease of MS). On the contrary, early plaques primarily present with microglial activation [13,65,91, 112]. Besides, early cortical lesions are associated with inflammation of the meninges [65]. Interestingly, such microglial activation, accompanied by infiltration by macrophages, can also be seen in the settings of several protozoan infections of the central nervous system [11,19, 60]. Besides, meningoencephalitis, although histologically different from that seen in MS, can be found in humans infected with trypanosomes [20].

It is also known that MS plaques are preferentially found in the parts of the brain containing high amounts of iron [1, 22,40]. Such a link may be indicative of viral infections, since some viruses selectively infect iron-acquiring cells. Still, the excess of iron within MS plaques seems to be located extracellularly, thus pointing against a role for viruses. Interestingly, *T. cruzi* requires an iron source for an optimal growth rate, and in hypoferremic hosts, the parasite is much less pathogenic [7,55,63,76]. Perhaps, other parasitic protozoa are also iron-dependent.

8. Which suspected microorganisms cannot be causing MS?

Epidemiological studies suggest that MS may be caused by an infectious factor. Yet, in spite of intensive research, no definitive evidence has emerged to identify a single microorganism that is unequivocally responsible. Since such a microorganism needs to meet several criteria

from histological and biochemical studies (lymphocytic composition of perivascular and parenchymal infiltrate responses, as well as cerebrospinal fluid characteristics), this excludes most bacteria and viruses, with their typical pyrogenic activity. However, it is possible that MS emerges as a result of so-called molecular mimicry [115]. Epstein-Barr virus (EBV) has long been suspected to be such a causal factor. It is known that MS is found more often in EBV-seropositive individuals compared with those who are seronegative. MS patients have also been found to reveal increased immune responses against EBV [8,45]. Besides, circulating EBV-specific CD8⁺ cells can be found in these patients. However, EBV infection is very common in China and Japan (infection rates in these countries are even higher than in the West) [108]; still, MS prevalence in these countries is low, pointing against a role for EBV in MS pathogenesis. Moreover, recent studies that have used RNA in situ hybridization and real-time polymerase chain reaction (PCR) assays have revealed that EBV is very unlikely to be a direct trigger of MS [85]. A similar role in MS pathogenesis, with the same unconvincing results, has been considered for other microorganisms: *Chlamydia pneumoniae*, measles virus, adenoviruses, respiratory syncytial virus, human herpes virus-6, parainfluenza viruses, and others. Yet extensive microbiological studies have failed to demonstrate any consistent elevated antibody titers to all those microorganisms studied [42,81]. Similarly, epidemiological studies, especially migrant data or other aspects of MS epidemiology, are not supporting the idea that these microorganisms could be responsible for triggering MS [8].

9. What do pharmacological trials tell us?

The currently ruling paradigm is that MS is an autoimmune disease. Consequently, therapeutic strategies predominantly target the inflammatory cascade or modify the immune response. Interestingly, only a fraction of chemical compounds that exhibit such immunosuppressive or immunomodulatory activity and have been proven to be effective in experimental autoimmune encephalomyelitis have also revealed beneficial effects in humans [18]. These drugs, recommended for the treatment of MS, comprise interferon-beta, glatiramer acetate, and mitoxantrone. It is thought that all these drugs decrease or modify autoimmune reactions in the central nervous system. Then, if MS were actually driven by an infectious agent and not by autoimmunity, one should expect a deleterious clinical effect of such immunoaltering drugs, which is not the case. Maybe the aforementioned pharmaceutical agents are not active through immunomodulation but rather through antimicrobial activity. Indeed, all of them—interferon-beta [48,49], glatiramer acetate [54] and mitoxantrone [29]—in addition to their immunoaltering properties, exhibit antiprotozoan activity. Besides, it should

be mentioned that some other drugs that are used for symptomatic treatment of MS—tetracyclines [77,78,119] and naltrexon [23]—also exhibit antiprotozoan activity [5, 46]. For the time being, the antiprotozoan efficacy of a new anti-MS drug, natalizumab, has not been studied. Perhaps such research should be performed. The other new drug, fingolimod, increased the susceptibility to *T. cruzi* infection in an animal experiment, elevated parasitemia, and accelerated the mortality of infected mice [30]. However, another study has shown that fingolimod modified lipid metabolism and protected mice from high-fat-diet obesity [47]. Thus, maybe this pharmaceutical agent, in addition to its known immunoaltering effect, is effective in MS through the modification of lipid metabolism.

A well-known latitude paradigm of MS may also point toward microorganisms. Vitamin D, which is suspected to be responsible for the unique geographic distribution of MS, may also be involved. Still, its role is not necessarily related to its immunomodulatory property, as usually thought. It has been found that high levels of vitamin D exhibit a protective effect against *T. cruzi* and *Plasmodium falciparum* [37,98, 114]. If MS were caused by a similar protozoan, a lot of sun, resulting in an adequate vitamin D level, might be protective against MS through its antimicrobial effects. Still, it should be remembered that vitamin D appears to contribute in defense against a number of viruses as well [120]. Besides, other light-dependent mechanisms may play a role (interestingly, apicomplexan and kinetoplastid protozoa are equipped with an organelle that probably developed from the chloroplast yet is no longer capable of photosynthesis). Some of these parasitic protozoa seem to be light-susceptible through melatonin-driven mechanisms, and light suppresses their replication [66]. Perhaps a hypothetical agent of MS exhibits similar properties.

10. Biological properties of the hypothetical microorganism

In summary, MS may be a rare complication of a highly prevalent (at least in high-risk areas) infection that is low or asymptomatic in the majority of infected individuals [53, 117]. Most likely, the microorganism responsible is also infecting other mammals, and probably such an infection in animals is also asymptomatic. Epidemiological, histological, and clinical studies on MS suggest that if the disease were caused by an infectious agent, such a microorganism should exhibit several unique properties: it (i) is transmitted by an arthropod vector; (ii) should be characterized by specific metabolism of lipids; (iii) should be dependent on iron; (iv) should be associated with autoimmune responses of the host; and (v) should be susceptible to interferon-beta, glatiramer acetate, mitoxantrone, tetracyclines, and naltrexone but not to the degree that would allow its eradication.

In addition, this hypothetical microorganism, at least in the chronic stage of the infection, should exhibit tropism toward the brain. Although some of these properties may suggest very different microorganisms, a combination of them is unique for protozoa, especially trypanosomes and other blood-residing unicellular parasites. Of course, such an infectious agent of MS has not yet been identified. But it is also known that standard histological examinations cannot reveal many protozoa [14,20,60,93,111], especially in the chronic phase of the disease. Tissue samples of MS patients have never been studied using immunohistochemical staining against protozoan antigens, and neither has a search for protozoan antigens been performed using PCR assays.

As has already been suggested in this paper, Scandinavian reindeer may be the primary animal reservoir of this elusive agent of MS. Indeed, reindeer are infected by a legion of different unicellular parasites: trypanosomes (*Trypanosoma cervi*, *Trypanosoma theileri*) and apicomplexans (*Babesia divergens*, *Babesia capreoli*, *Besnoitia sp.*, *Eimeria sp.*, and *Sarcosporidia sp.*) [92]. Probably, other species of parasitic protozoa infecting these animals are still undiscovered. Faroe Islands studies suggest that the first contact with this hypothetical infectious agent of MS may manifest with acute gastroenteritis [117]. This may mean that the microorganism is attacking the intestines. Perhaps, if it is actually fat-dependent, as suggested by some epidemiological studies, it is initially residing in the intestinal lymphatics, with easy access to the lipid-rich chyle. Some parasitic trypanosomes preferentially invade and replicate in the lymphatic vessels of the abdominal cavity [90,103]. If such a scenario is correct, then the microorganism causing MS, after replication in the intestinal lymphatics, should spread into the bloodstream through the thoracic duct. This may account for the high prevalence of malformed valves of the left internal jugular vein seen in MS patients [101], since the thoracic duct is joining the venous system in proximity to the left jugular valve.

There are, however, some data pointing against a potential role for trypanosome-like protozoans in the pathogenesis of MS. Epidemiological studies on MS suggest that children under the age of 15 are resistant to this disease [24,52,73]. Still, trypanosomes are usually more virulent in children [79,96]. This means that trypanosomes (at least those exhibiting a similar biology to African and American species) are unlikely to be responsible for MS. On the contrary, another genus of parasitic protozoa, *Babesia*, is characterized by selective infection of adult hosts. Young animals, as well as young humans, are protected against these parasitic apicomplexans through innate immunity mechanisms [38,57,61,110,113]. Interestingly, proper function of the spleen is needed [38,113], and splenectomy of immature animals abolishes their resistance against

these parasites [57]. It is well known that MS is rarely seen in children. Then, if such a low prevalence of MS in children were due to a similar mechanism as in the case of babesiosis, one should expect a higher prevalence of MS in those children who underwent a splenectomy. Yet such a surgical procedure is rarely performed in children, and no published evidence supports this idea. There is, however, an intriguing report on comorbidities in children suffering from immune thrombocytopenia. This pathology is associated with splenic dysfunction. Of note, an unexpectedly high prevalence (25 times more than anticipated) of pediatric MS has been found in this particular group of children [97].

11. How to test this hypothesis?

In order to test this protozoan hypothesis of MS, extensive research should be performed. It should be expected that simple blood smears will be negative. The microbe, if it really exists and inhabits the vascular system, is probably not available for standard screening, since it is residing in atypical locations (like the intestinal lymphatics and cerebral venules). It is also possible that after the first episode of infection, it is no longer present in the bloodstream but escapes into immunoprivileged organs (a specific intrathecal immune reaction in MS patients suggests a response against such brain-residing microorganisms). Moreover, it should be remembered that standard tissue staining may be inadequate to reveal many protozoa [60,93,111]. Rather, such techniques as immunohistochemical staining and PCR assays should be used. Yet with the microbe not yet known, this will not be an easy task. Modern diagnostic techniques, like multiplex molecular technology, should probably be used. Such molecular probe technology does not require growth of a microorganism in culture. The test requires only a sequence of sequential bases unique to the genome of a pathogen of interest. Perhaps, a screen should be performed using the antigens of known parasitic protozoa that infect domestic and wild animals in high-risk areas for MS. There are also some common protozoan epitopes that could be used in such research [44,83,105].

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