Hygiene hypothesis: Innate immunity, malaria and multiple sclerosis

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Summary The establishment of new hygienic conditions plays a role in the appearance of autoimmunity in “westernised” countries. Consistently, but still unconvincingly, several epidemiological and immunogenetic evidences link the disappearance of malaria with the increase of multiple sclerosis (MS) in Sardinia, insular Italy. To this purpose, we have made an attempt to build a relationship between malaria disappearance and MS under the light of the hygiene hypothesis. This relationship has taken into account the MS frequency increase soon after malaria eradication in Sardinia, the present malaria endemism in Africa, the innate immune system activity here represented by Chitotriosidase (Chit), an hydrolytic enzyme produced by macrophages, and an unproductive polymorphism of Chit gene (CHIT1) as a measure of the genetic weight of Plasmodium-related immunity in these populations.

Data were derived from both experimental results specifically designed for this study and other data obtained from the available literature. The experimental and the hystorical–epidemiological findings concur to indicate that whilst in Africa CHIT1 mutation is rare and MS incidence is very low due to unmodified parasitic influence and hygienic conditions, in Sardinia a relationships between CHIT1 mutation, plasma Chit activity and MS prevalence rate is detected, even to a higher extent compared to Sicily, area at former lower rate of malaria endemy.

Upon such a basis, we have found convincing argumentations that, at least in part, MS has increased over the last four decades in Sardinia also because of the eradication of malaria, 50 years ago. This infectious disease that run for centuries in Sardinia, besides well documented enzyme deficiencies and red cell pathologies, have left an abnormal macrophage reactivity against Plasmodium falciparum. As a result, some Sardinian individuals secrete abnormally high levels of mediators of the innate immunity, relics of former protective anti-malaria infection, in response to new environmental factors. Therefore, MS, an immune-conditioned pathology of the central nervous system has been subject to an unexplained epidemiological increase in the last few decades in Sardinia because cells of the innate immune system, immuno-genetically selected over the centuries in response to widespread P. falciparum
malaria, have kept the tendency to over-respond to triggering factors even after the disappearance of malaria. This hypothesis may have an influence in re-directing clinicians toward a innate immunity-based rather than an antigen specific-based new MS therapies.

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Introduction

The association between a reduced exposure to infectious agents as a part of a changed lifestyle and a higher prevalence of atopy and autoimmune disorders seems now to be confirmed by consistent evidences [1,2]. However, the mechanisms underlying this association are not yet completely clear and could be attributed to both immune deviation and altered immune regulatory mechanisms [3]. The revolutionary ''hygiene hypothesis'' has received numerous more or less subtle modifications by different researchers in the fields of epidemiology, clinical science, and immunology [4]. Various pieces of a complex interplay between immune responses of the host, characteristics of the invading micro organism, pressure of the environmental exposure and interactions with a genetic background have now appeared [5].

Multiple sclerosis and the hygiene hypothesis

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS), which develops in young adults with a predisposing genetic trait and probably requires an environmental insult, such as a viral infection, to be triggered [6]. Its geographical distribution usually describes areas of high (northern Europe), medium (Mediterranean basin) and low prevalence rate (Africa) [7]. While in the Italian island of Sicily, a recent survey indicates a rather stable mean MS frequency over time [8,9], our size-comparable repeated studies in Sardinia, insular Italy, indicate that MS incidence and prevalence has dramatically increased in the last 40 years resulting in a rate which is one of the highest in the world despite its Mediterranean location [7,10]. Little is known about the cause of this rare phenomenon which represents the focus of the present study.

Stepping a little backward, a large body of evidences indicates that both allergic and autoimmune diseases, including MS, are rare in Africans, but dramatically increased in frequency and severity as soon as Africans move to ''developed'' countries such as USA [11–14]. Undoubtedly, this phenomenon should be interpreted as caused by an environmental change. In Sardinia, profound environmental modifications (among all, the eradication of malaria has been the wider and faster) have indeed occurred after the second World War, the extent of which resembles the environmental change of migrant populations. Perhaps not coincidentally, several epidemiological observations now link malaria and MS after only 50 years after hygienisation, providing a reasonable explanation for the explosion of MS cases in Sardinia. These evidences (reviewed in Refs. [6,15]) include: the presence of a spatial and temporal cluster of MS occurred soon after malaria eradication [16]; the observation that the A30-B18-DR3 HLA haplotype (the strongest MS-associated haplotype in Sardinia) shows the highest odds ratios in the same high malaria prevalence areas; the evidence that some tumor necrosis factor (TNF) polymorphisms in the TNF-promoter region already associated with both malaria and MS are up to 10 times elevated amongst Sardinians as compared to Sicilians and any other population worldwide [17–20].

MS and innate immunity

The peripheral activation and subsequent migration in the CNS of autoreactive Th1 cells are said to be the initial events in MS, and these cells are probably important players in the long-term evolution of the disease at least in many cases [21]. Nonetheless, the damage of the target tissue (central myelin and axons) is mediated by other components of the immune system and, particularly, by factors produced by the innate immunity [22]. In fact, despite a pathogenic role of T cell-mediated adaptive immunity is advocated, the presence of activated infiltrated macrophages and resident microglial cells represent a common pathogenic denominator in most MS lesions and these cells strongly contribute to MS brain damage through a group of neurotoxic factors [21–24].

Chitotriosidase, MS and malaria

In this context of hyperproduction of soluble innate immune mediators, the macrophage-derived chitotriosidase (Chit) is clinically relevant. A significant increase of plasma and cerebrospinal fluid Chit levels has been described in strict correlation with the clinical severity of MS [25] and apparently
more strictly correlated with the outcome as compared to other markers of macrophage activation such as TNF-α. Furthermore, Chit production is compartmentalised within the CNS, which confirms microglia and infiltrating macrophages to have a determinant role in the pathogenesis of the neurological damage [25]. Chit is a component of the innate immunity that plays a role in defence against chitin-containing pathogens. Its level increases during macrophage activation [26] and in chronic conditions such as Gaucher disease, β-thalassemia [27], and particularly, in parasitic infections such as Plasmodium falciparum malaria [28]. A 24 base pairs duplication in its exon 10 causes an asymptomatic Chit activity deficiency in about 6% of Caucasian population [26], 5.5% individuals from Sicily (44% heterozygosis) and 3.7% (33% heterozygosis) individuals from Sardinia [29]. On the contrary, this CHIT1 mutation is absent in sub-Saharan Africa areas, endemic for P. falciparum malaria and other parasites [29].

Hypothesis

This observations leads us to consider the idea that, contrary to Africa, Chit is not longer useful in the “hygienised” Italian islands for the defence against chitin-containing parasites, and it either only represents a trace of a dismissed ancestral macrophage response or it may hide some unknown immune functions. Aim of this study is to be able to build a relationship between malaria eradication and MS increase under the light of the hygiene hypothesis. This relationship will take into account four protagonists: (i) the MS frequency increase soon after malaria eradication in the “westernised” Italian island of Sardinia, (ii) the present malaria endemicity in Africa, (iii) the innate immunity against malaria, here represented by the determination of plasma Chit levels and (iv) the inactive polymorphism of CHIT1 as a measure of the genetic weight of plasmodium-related immunity in these three populations.

To this purpose, data for constructing the hypothesis are both new experimental results specifically obtained for this study and data obtained from our own published studies or other available literature.

Subjects and methods

Chit determination and subjects

Plasma from 107 healthy individuals of Sardinian ancestry, 55 females and 52 males, mean age 30.7 years (range 20–40), were collected from the Sassari Blood Transfusion Center and from the personnel of the Institute of Clinical Neurology, University of Sassari.

As for the Africans, controls included 149 African subjects from Ouagadougou, Burkina Faso, Sub-Saharan West Africa (81 males and 68 females), mean age 30.5 (range 20–42). Subjects had neither serious underlying illness nor hematological disorders such as haemoglobinopathies, leishmaniasis or infectious diseases known to influence plasma Chit levels. They had neither signs of acute infectious diseases nor positive P. falciparum smears. All subjects were negative for HIV antibody testing.

Sicilian controls included 331 healthy subjects, 175 males and 156 females, mean age 29 (range 20–41), selected from the Catania Blood Transfusion Centre. All subjects gave informed consent to participate in the study according to the Helsinki Declaration.

Chit activity was measured in plasma by incubating 5 μl of undiluted plasma with 100 μl of a solution containing 22 μmol/l of the substrate 4-methylumbelliferin-β-D-1,4-N,N′,N″-triacetyl-chitotriose (Sigma Chemical Co.) in 0.5 M citrate–phosphate buffer, pH 5.2, for 15 min at 37 °C. The reaction was stopped by using 2 mL of 0.5 mol/l Na2CO3–NaHCO3 buffer, pH 10.7. The fluorescence was read on a Hitachi 2500 fluorimeter, on 365 nm excitation and 450 nm emissions. Chit activity was measured as nanomoles of substrate hydrolyzed per ml per hour (nmol/ml/h).

Prevalence of CHIT1 mutation

In order to compare the homozygous and heterozygous frequency for CHIT1 duplication of 24 bp in exon 10, that causes a Chit activity deficiency, results from African, Sicilian and Sardinian populations were extracted from our study [29].

Prevalence of MS

Our data on MS prevalence in Sardinia (~150 per 105 population) are taken from Refs. [7,10,16], those for Sicily from two recent works conducted in two distinct areas of this island (Palermo and Catania), which found mean MS prevalence rates of 71 and 58.5 per 105 population, respectively [8,9]. MS frequency in Burkina Faso has never been studied and we conventionally attribute it a mean prevalence rate <5 per 105 typical of African countries where the disease is known to be very rare [30].
Statistical analysis

Statistical analysis was performed by using the SigmaStat 3 Software. Two-tailed Student’s t-test and single regression analysis were used as required. Significance was conventionally established for p values <0.05.

Results

Plasma Chit activity

The average Chit activity in African plasma donors was 15.6 (5.1 SD) nmol/ml/h, whereas in Sardinian and Sicilian donors the values were 9.3 (2.6 SD) and 4.6 (1.9 SD) nmol/ml/h, respectively (Fig. 1). The differences of Chit activity level between the three populations were extremely significant: Sardinians vs. Africans p = 0.008, Sardinians vs. Sicilians and Africans vs. Sicilians p < 0.001 for both (Fig. 1).

Regression analysis

Plasma Chit activity level amongst Africans, Sardinians and Sicilians were correlated with the percentage of the non-active CHIT1 polymorphism in the same populations. The linear regression coefficient was —0.98 for the CHIT1-deficient population at the heterozygous and —0.99 at the homozygous state. Nevertheless, the p value was not obtained because of the few data points (Fig. 2).

MS prevalence, Chit level and CHIT1 frequency

Fig. 3 shows that the plasma Chit activity is directly proportional to the MS prevalence rate (per 10^5 population). In Africa, MS is substantially absent despite the high level of plasma Chit activity (hygiene hypothesis). Values are reported in a logarithmic scale on Y axis.
population (found in Sardinia and Sicily (see "Discussion"). In Africa, where higher level of plasma Chit activity is observed, the MS frequency is negligible quite possibly as an effect related to the actual malaria endemism.

Discussion

Mechanisms of the hygiene hypothesis

The "hygiene hypothesis" suggests that an environment rich in normal microbial flora primes the immune system in the Th1 direction, while a "sterile" environment promotes the development of pathological immune phenotypes against self antigens [1–5,31]. Cellular and molecular mechanisms include a fine-balance between innate immune mechanisms and Th1–Th2 development, and their interaction with gene polymorphisms of the immune system. However, the "hygiene hypothesis" continues to pose numerous questions concerning the nature of protective infectious agents, the timing of their involvement with regard to the natural course of a given immune diseases and, most importantly, the mechanisms of protection that infectious agents would exert against the (auto)immune disease itself. Some arguments seem to be convincing as reviewed in Ref. [3]: a hypothesis deals with the immunoregulation, i.e., an infectious agent stimulates a variety of modulatory cells (Th2, T regulatory CD25+, NKT); another hypothesis identifies no antigenic components of infectious agents which may stimulate cells of the immune system through mechanisms which are not recognized as antigenic. A third hypothesis deals with a chronic and low microbial stimulation early in life, a mechanism which could lead to a weaker Th1 response and a stronger Th2 response. Finally, others suggest an antigenic competition, i.e., the immune responses against pathogens compete with the appearance of immune response against self antigens (autoimmunity). This is probably an important mechanism but its modalities are still elusive.

Malaria hypothesis in Sardinia

With no doubts the Sardinian genome has been subjected to selective pressures to resist to malaria in spite of a heavy segregational load. As a few examples, thalassaemia and glucose-6-phosphate dehydrogenase deficiency have been considered an efficient genetic protection against *P. falciparum* malaria and this mechanism has expanded these two genetic anomalies in the island [32]. Consistently, our recent results [33] confirm that a correlation exists between the strongest anti-*Plasmodium* immune response [34] and the MS condition among Sardinians subjects [33]. This finding tends to demonstrate that genetic traits that may have been selected in Sardinia for conferring protective responses to *P. falciparum* are now closely linked to alleles predisposing to the pathogenesis of MS. Accordingly, the average plasma Chit activity and the MS prevalence in Sardinia are higher than those found in Sicily, perhaps as a reflection of the distinct malaria-induced genetic pressure in these two populations. The difference between Sardinia and Sicily may be correlated to both the different degree of plasmoidal diffusion at the population level and the different species of *Plasmodium* circulating in each islands which had corresponded to significantly different mortality rate for malaria in the period 1887–1889 being $298 \times 10^5$ in Sardinia and $142 \times 10^5$ in Sicily, respectively [35]. Also, MS prevalence rate is inversely correlated to CHIT1 gene polymorphism, both at the homozygous or heterozygous state in the populations of Sardinia and Sicily. On the contrary in Africa, the high prevalence of malaria and many other parasitic diseases preserves the wild CHIT1 gene and maintains high the level of the innate immunity. In evolutionary terms, the loss of lytic activity of CHIT1 in people living in developed countries suggests that, given the improved social and environmental conditions and the progressive lack of chitin-containing pathogens, the wild CHIT1 gene in these countries may be functionally redundant and then dismissed as relict of evolution [36]. In Sardinia however, even in the absence of parasitic infections, we found a lower rate of CHIT1 mutation as compared to other Caucasians [29], including Sicilians, thus leading to higher Chit level, which may represents a reminder of a not yet dismissed macrophage hyperactivity against malaria.

Implication of the hypothesis

A dramatically rapid change of the environment after the eradication of malaria has allowed the Sardinian immune system to abnormal responses (built up with the aim of ensuring an strong anti-*Plasmodium* response) after the contact with inciting and triggering environmental insults [33]. As for MS, the question whether Th2 and Th1 response are increased or diminished is perhaps a matter of inconsistency. Our findings in fact are a step backward the initiation of an antigen specific response and, they seem to support the working idea that the innate, antigenically unrelated responses are skewed in the population of Sardinia. On the basis
of epidemiological evidences and the new laboratory findings, we have serious arguments favoring the idea that MS incidence increased over the last four decades in Sardinia also because of the eradication of malaria, 50 years ago. This infectious disease has left an abnormal macrophage reactivity against Plasmodium [33].

Based on a evolutionary perspective and on the principles of the hygiene hypothesis, we think that immune cells of certain Sardinian individuals are forced to abnormally high and auto-directed immunity in response to new environmental factors. This phenomenon may arise in the absence of a strong and “competitive” immune trigger such as Plasmodium. This hypothesis may have an influence re-directing clinicians toward a innate immunity-based rather than an antigen specific-based new MS therapies [37]. Consistently, a therapeutic immunoregulatory effect has been documented when the (Bacillus Calmette-Guerin) BCG was administered to MS patients [38]. This may support the idea that an immune deviation occurs through a competitive interaction between strongly antigenic and “dangerous” environmental signals and the innate immune system.

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References


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