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Review

Does the "Hygiene Hypothesis" Provide an Explanation for the High Prevalence of Multiple Sclerosis in Sardinia?

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The "hygiene hypothesis" describes a hypothetical scenario in which the balance between T_H1 (defending host against bacterial and viral infections) and T_H2 (defending against parasitic infections) immune responses is pivotal and in which the consequence of reducing the infectious stressors during infancy is increased autoimmunity (T_H1-mediated) and allergy (T_H2-mediated). Many epidemiological observations confirm that allergic and autoimmune diseases are significantly increased in the "developed" countries and negatively associated with childhood infections. However, it has been recently revealed that immune elements associated with allergy are extensively involved also in the pathogenesis of autoimmune demyelination and that T_H2- and T_H1-mediated infections ameliorate the course of the disease confirming that the allergic root is also responsible for the escalation of autoimmune disorders, and both have a common immunological denominator.

In the Italian island of Sardinia, MS and type-I diabetes frequencies have sharply increased in the last decades compared to other populations living in the same Mediterranean area. Initial observation led us to believe that environmental changes favoured the MS risk rise, thus sustaining the hygiene hypothesis. However, data on MS prevalence distribution in this territory suggest that other mechanisms than environment have also to be taken into great account. Our recent epidemiological studies reveal significant differences in the MS prevalence between rural and urban areas within the same province of Sassari but, contrarily to what expected from the hygiene hypothesis, MS prevalence is significantly higher in rural, genetically "archaic", areas where the westernalization process has been less pronounced. On this basis we believe that, beside hygiene-related factors, genetics could represent a more relevant determinant of Sardinian high susceptibility to MS.

Keywords: Hygiene hypothesis; Multiple sclerosis; Sardinia

THE HYGIENE HYPOTHESIS

There has been a significant increase in the frequency of allergic and autoimmune diseases over the past three decades in the so called "developed" word, whilst their prevalence is considerably lower in "developing" countries. Nonetheless, epidemiological studies show clear differences in the prevalence of allergies between rural and urban areas within the same country.[1] Explanatory observations suggest that environmental factors clearly associated with industrialization and urban living, such as pollution, changes in diet and breastfeeding, reduced exposure to outdoor UV lights and increased exposure to indoor allergens could only poorly account for this dramatic rise. On the contrary, researches conducted on other environmental factors, namely the childhood infections, seemed to show an overwhelming negative association with autoimmune and allergic diseases, suggesting that the more and earlier infections the more protective effect.[2,3] It can be speculated that the use of antibiotics and vaccination (and the generally improved hygiene) leading "developed" children to reduced bacterial, viral and parasitic infections, led to insufficient or abnormal stimulation of immune responses. Hypothetically, a high prevalence of chronic infections educates the immune system towards a regulatory network which, in turn, is weakly developed in industrialized countries characterised by a low pathogen load, allowing pathological immune reactions even to innocuous (or "self") substances.

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This so-called "hygiene hypothesis"[2] is based on an immunological scenario in which the balance between type 1(T_H1, defending host against bacterial and viral infections and leading to autoimmune diseases) and type 2 (T_H2, defending host against parasitic infections and virtually leading to allergic diseases) immune responses is pivotal.[2,3] In terms of evolutionary perspectives, the logical consequences of reducing the infectious stressors that have guided the development of the immune system for millennia are on one hand reduced child mortality and increased longevity but on the other hand increased allergy-autoimmunity. This immunological explanation has been influential in shaping strategies to prevent allergic diseases.[3] A recent study suggests a crucial role of environmental exposure to endotoxins in promoting tolerance to allergens in natural environments.[4] Also, the induction of T_H1 responses by Bacille Calmette-Guerin (BCG) is being advocated in the treatment of multiple sclerosis (MS)[5] a chronic demyelinating disease of the central nervous system of putative autoimmune, T_H1-mediated, origin.

More recently, allergic and autoimmune diseases have been epidemiologically and experimentally associated. The incidence of type-I diabetes, a paradigmatic T_H1-mediated disease, has been progressively increasing in association with asthma (T_H2-mediated) at the population level.[6] In the animal model of MS, experimental autoimmune encephalomyelitis (EAE), it has been recently revealed that immune elements associated with allergy are extensively involved in the pathogenesis of autoimmune demyelination, suggesting that the pathogenesis of MS must now be viewed as encompassing elements of both T_H1 and T_H2 responses.[7] Helminth (T_H2 inductor) and mycobacterium (T_H1 inductor) infections also ameliorate the course of EAE confirming that the hygiene hypothesis may apply to autoimmune demyelinating diseases.[8] This should suggest that the allergic "denominator" is contextually responsible for the escalation of autoimmune disorders which cannot merely explained by the T_H1 vs. T_H2 imbalance, but instead should have a common, so far obscure, immunological root.

THE "HYGIENE HYPOTHESIS" IN SARDINIA

In the genetically distinct and homogeneous population of Sardinia, MS as well as type-I diabetes incidences have dramatically increased in the last four decades compared to other Caucasian and non-Caucasian populations living in the same Mediterranean area. As a result, Sardinians bear the unenviable distinction of having among the highest incidence rates for type-I diabetes and for MS in the world.[9,10] Also, a co-morbidity of MS and type-I diabetes in this island seems to be extremely peculiar, which has driven researchers to speculate on common susceptibility factors related to the pivotal role of the HLA association.[11]

We formerly reported of a temporal cluster of MS in Sardinia which seemed to occur after the breaking of geographical and historical isolation (Fig. 1) leading to improved sanitation and nutrition, malaria eradication and diffuse vaccinations (in two words, the "westernalization process").[12,13] In more recent studies,[9] by comparing our methodologies and results vs. those obtained in Ferrara (Northern Italy) and in Iceland, we have formally excluded that improvement of diagnostic accuracy could have been responsible for the greater number of MS cases in Sardinia (Fig. 2) and confirmed that Sardinia is possibly considered a "MS focus". Since a forty-year span is too short for a substantial change in Sardinians' genetic pool to occur, an environmental change should be assumed favouring the view that the MS risk rise is sustained by the hygiene hypothesis.

However, more recent data on MS prevalence distribution in our territory suggest that other mechanisms than environment have also to be taken into great (or greater) account. Gene-frequency analysis of Sardinians shows heterogeneity at the micro-geographic level which correlates with linguistic and archaeological evidences

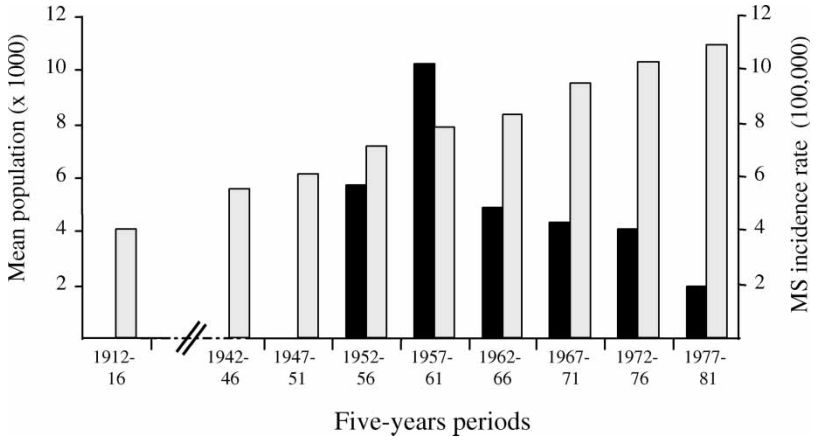

FIGURE 1 Average annual incidence rates (per 100,000 per year, right Y axis) of MS in Macomer, Sardinia, from 1912 through 1981 (5-years periods). Left Y axis expresses the population growth (per 1,000). Adapted from reference.[12]

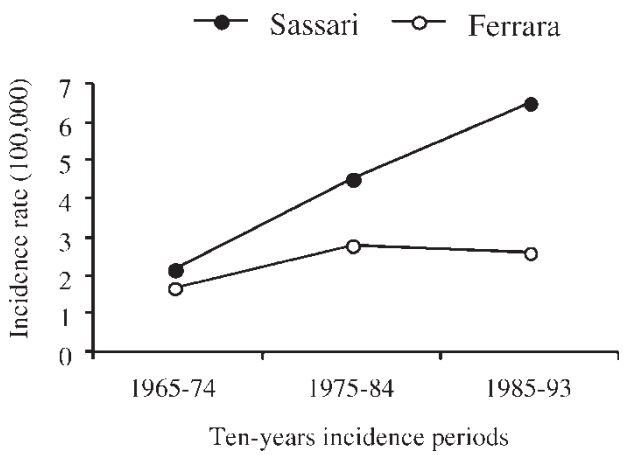

FIGURE 2 MS incidence trend in Ferrara (northern Italy) and the province of Sassari in comparable time periods and using comparable methods of MS case ascertainment and diagnosis. Adapted from Ref. [9].

(Fig. 3a; reviewed in Ref. [13]). In this context of micro-geographic heterogeneity, our epidemiological study showed significant differences in the prevalence of MS between rural and urban areas within the same province of Sassari.[14] However, contrarily to what expected from the hygiene hypothesis, we found that MS prevalence is significantly higher in areas where farm animals and related activities are more diffuse which correspond with genetically and linguistically more preserved areas, and lower in genetically mixed areas where the westernalization process has been predominant[14] (Fig. 3b).

On this basis, we believe that, beside hygiene-related factors, genetics could represent a relevant determinant of Sardinian susceptibility to MS and, as already pointed out by Weiss in his editorial,[15] eating dirt or moving to a farm is hardly protecting our population toward the risk of autoimmune diseases.

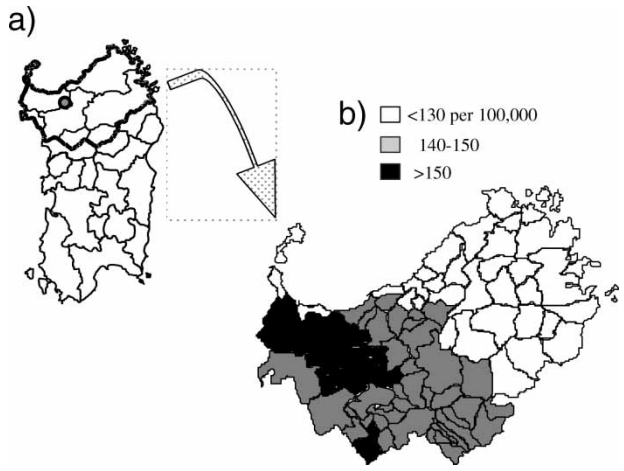

FIGURE 3 (a) Province of Sassari, northern Sardinia, in bold line. Lines configure the micro-geographic subdivision according with significant genetic differences. Adapted from Ref. [13] (b) mapping of the distribution of prevalent MS cases per 100,000 population in the all communes of the province of Sassari (approximately 454,000 population), on Dec 31st, 1998; the MS distribution gradient is evident being the southern–western part of the province significantly at higher MS risk. Adapted from Ref. [14].

CONCLUSIONS

The experimental method, which involves making predictions and testing them in controlled laboratory settings, is commonly said to be the only paradigm for testing hypotheses. Unfortunately, not all scientific hypotheses can be tested in a laboratory. Historical researches explain observable phenomena in terms of causes that are unobservable or that cannot be reliably replicated in a laboratory setting. This kind of researches are usually associated with scientific fields such as paleontology or planetary science, though some good examples in medicine are provided (reviewed in Ref. [16]). In this paper we try to analyze the reasons that may link the recent significant increase of autoimmunity, in particular MS, in Sardinia in the light of historical changes and epidemiological "experimental" advances.

Childhood infections in developing countries clearly outweigh that in the developed world. However, direct comparisons are complicated by a plethora of biasing variables and, though the general agreement is that protection from allergy-autoimmunity is associated with increased microbial exposure, the link has remained indirect. The unexpected explosion of asthma cases in the inner, more rural, cities of the United States[17] provides an example counterpoint to the hygiene hypothesis. In retrospective studies conducted in the tropics,[18] vaccinations with BCG were associated with a reduction of allergies being the earlier the vaccination, the greater the protection, which was not observed in Swedish children even in the presence of a family history of allergy.[19]

In the case of MS, the picture is even more complicated. Today MS seems not to delineate a unique disease, but rather a spectrum of disorders with several biological variations. Different immuno-genetics may lead to distinct mechanisms of destruction of myelin sheaths and axons: in some patients lesions are dominated by T-cell and macrophage-mediated demyelination while other patients have preferentially B cell and complement-mediated mechanisms or even a primary oligodendrocyte defect.[20] More recently, allergic and autoimmune elements are described to be co- involved in the pathogenesis of autoimmune demyelination.[7] Finally, the dichotomy between T_H2 and T_H1 cytokines in MS patients is no longer paradigmatic since up-regulation of pro- or anti-inflammatory cytokines appears to evolve simultaneously in most patients.[21]

In conclusion, to date the application of hygiene hypothesis in Sardinian MS does not provide an imbalance towards environment in the known paradigm assuming that environment is equally influential in the context of genetic susceptibility corresponsible factors. It is likely that common external events, possibly involved in the initial pathogenic event leading to the MS appearance, occur within a complex and more influential interaction of gene loci in the genetically homogeneous population of Sardinia. Even though in other areas, in which

the westernalisation process and the exposure to "developed" environment has almost paralleled ours, the MS increase is also documented, that in Sardinia is not only much higher but also delineating a dramatic problem of socio-economic impact.

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