

INTRODUCTION

It is a concept based on the premise that reduced exposure to microorganisms would result in increased immune reactivity promoting allergic and autoimmune disorders. This has been evident in Western countries where high quality of sanitation has drastically reduced exposure of the population to micro-organisms presumed to have contributed to the 'silent epidemic' of autoimmune disorders in first world countries. This premise is supported by epidemiological, experimental and clinical evidence. Incidence of asthma has increased significantly in children in the last 3-4 decades and similarly there is increased prevalence of inflammatory bowel disease (IBD), Type-1 diabetes (T1D), multiple sclerosis (MS) in the past 50 years. Interestingly, such increased incidences of allergy and autoimmune diseases have not been reported in low-income tropical countries where infections are most frequent indicating a possible reciprocal association between infection and allergy/autoimmune diseases which forms the basis of hygiene hypothesis (HH).

EPIDEMIOLOGY

The concept of 'Hygiene Hypothesis' (HH) was for the first time introduced by Strachan in 1989 based on the observation of an inverse correlation between sibling size and risk of allergy: more the sibling sizing less was the chance of developing allergic disorders. This was further validated in other studies in different populations. Furthermore, a meta-analysis of 32 studies revealed decreased risk of atopic dermatitis in infants or children exposed to pet dogs, those consuming less antibiotics and in those who grew up in rural areas.

Besides susceptibility to allergy, the HH is also valid for autoimmune diseases. Interestingly, persons from similar ethnic background living in two different environmental conditions showed six fold higher prevalence of T1D in areas with good sanitary conditions. These observations have been attributed to varied infection rates in two diverse geographical areas: reduced exposure to infection increases autoimmune response to self, facilitating susceptibility to T1D. Prevalence of IBD is higher in US compared to sub Saharan Africa, and increased helminth infection in Africa has been attributed to play a modulatory role in preventing IBD. In India, an inverse correlation between filarial infection and autoimmune disorders such as systemic lupus erythematosus and rheumatoid arthritis has been observed. Interestingly, SLE is more frequent in African Americans compared to West African. Furthermore, human migration studies

have shown that migrants from areas with low incidence of autoimmune disease suffer more from these diseases when they migrate to higher incidence areas.

Allergy and autoimmune diseases result from an exaggerated host immune response against self antigens. Several factors such as genetic susceptibility, immune dysregulation and environmental triggers have been implicated in the causality of autoimmune diseases. Genetic factors are unlikely candidates for this surge since mutations associated with disease susceptibility requires a long time span to manifest and the observations on HH are only several decades old. Environmental factors, like parasite infections, play an important role in protection against allergy and autoimmune diseases since they modulate the host immune system for their own survival. Excessive attention to hygiene and sanitation has resulted in a parasite free environment that has resulted in loss of control over a hyperactive host immune system.

Experimental Evidence

The HH has been subjected to analysis through experimental models and there is robust evidence to support the premise enumerated earlier.

The beneficial nature of infections against different autoimmune disorders through their immunomodulating activity has been demonstrated in animal models. Murine model of colitis induced by trinitrobenzene sulfonic acid (TNBS) or dextran sodium sulfate (DSS) is significantly reduced after exposure to *S. mansoni* soluble egg antigen through enhancement of IL-4, IL-10 and reduction of IFN- γ . Furthermore, infection of mice with *T. spiralis* cercariae antigen significantly reduces severity of disease when colitis is induced subsequently.

The administration of helminthic products also showed promising results in experimental autoimmune encephalitis (EAE), a model for MS (multiple sclerosis). Before the onset of EAE, administration of *S. mansoni* antigen or its cercariae reduced the severity and incidence of EAE through decreased TNF- α , IL-12, IFN- γ and increased TGF- β , IL-4 and IL-10. In addition, transfer of splenic T-cells from *T. spiralis* infected rats into experimental model significantly protected them from disease onset.

In collagen induced arthritis (CIA) model, akin to RA, effectiveness of ES-62, a filarial parasite (*A. vitae*) excretory glycoprotein molecule has been established. ES-62 interfered with initiation, progression and severity of CIA by diminishing Th-1 and Th-17 immune responses.

146 Several experimental evidence indicates protection of non-obese diabetic (NOD) mice from rapid progression to manifest diabetes by infection with *S. mansoni* cercariae antigens through expansion of TGF- β and increased production of IL-10, IL-4 and IL-13, the anti-inflammatory cytokines.

CLINICAL EVIDENCE

Human trials of the effect of parasite products to protect or modulate autoimmune disorders has taken the fancy of the scientific community. Several clinical trials have been carried out to assess safety and effectiveness of helminth therapy. Clinical trials of *Trichuris suis* (TSO) in CD (Crohn's disease) and refractory UC (Ulcerative colitis) have revealed significant reduction of disease severity, increased number of responders and enhanced remission rate. However, a recent phase-2 clinical trial in 2013, failed to demonstrate effectiveness of TSO against CD. An important follow-up study comprising of (a) MS patients with naturally infected helminths, (b) MS patients without infections (c) helminths infected subjects and (d) healthy controls. The observation suggested that MS patients who harbored helminth infection had reduced disability scores, lower number of relapse and improved magnetic resonance activity imaging compared to MS patients without helminth infections. Investigation revealed higher levels of IL-10, TGF- β , the anti-inflammatory cytokine, and lower levels of inflammatory cytokines like IFN- γ and IL12 in infected MS patients compared to those without infections.

Although epidemiological data and experimental models suggest a possible therapeutic role for helminths or its products in RA and T1D there are no clinical trials using helminth derived products for therapy in these diseases. Some robust data from Indian population showed a significant negative association between filarial infection and autoimmune disease such as SLE and RA. Recently, a negative correlation of IL-17 and filarial antigen levels has been postulated as a mechanism of action of filarial worm products in reducing susceptibility to RA and SLE. But it remains to be seen if these observations translate into feasible application.

THE IMMUNOLOGICAL BASIS OF HYGIENE HYPOTHESIS

The immunomodulatory effects of helminth have been extensively investigated. It has several important properties including repression of T-helper (Th)1/Th17 differentiation, elevation of Th-2 cells, amplification of T regulatory (Treg) and B-reg cells, pivotal in maintaining immune homeostasis, and change of dendritic cells towards a more tolerogenic subset. Dendritic cells are vital in antigen presentation to T cells.

Naïve T-cells differentiate into Th or Treg cells based on their requirement. T-helper cells further polarize into Th1 and Th2 subsets having distinctly opposite function. Th1 response has been linked to the pathogenesis of autoimmune diseases cells through production of distinct proinflammatory cytokines. The Th2 response is linked to atopic disorders through increased production of IL-4, IL-5, IL-9, IL-10 and IgE. Experimental models infected

with helminths have shown a Th2 type immune response. Thus helminth infections should lead to atopic disorder in experimental models but it does not translate into disease manifestations. This indicates the importance of other factors such as Th17 and Treg in the final outcome of disease pathogenesis. Infection with helminths down regulate Th1/Th17 mediated inflammatory disorders through increased production and IL-10 and TGF- β . Furthermore, it enhances proliferation of Treg cells that regulates the balance between Th1 and Th2. Administration of ES-62 helminth product to a collagen induced arthritis model improves disease pathogenesis by suppressing Th1/Th17 cytokines, and increasing Th2/Treg cytokines (IL-4, IL10). Interestingly, filarial infection in human suppress IL17 production and possibly polarize immune response towards Th2 type.

Helminths promote proliferation of B regulatory cells (Breg) and increase production of IL10. Generation and proliferation of B reg cells by helminth infection have been reported in MS, IBD and CIA model and restoration of IL10 was observed in joints of CIA model

Dendritic cells play a pivotal role in the innate immune system and in autoimmune diseases. Important functions of dendritic cells are to capture, process and present antigen to T-cells. DC directs T cells polarization towards immunogenic (Th1, Th17 phenotype) or tolerogenic (Th2, Treg responses). Tolerogenic DC have beneficial role against autoimmune diseases. Polarization of DC towards tolerogenic phenotype has been stimulated by parasite products through toll like receptor or C type lectin receptors.

The other important cell of the innate immune system that drives the adaptive immune response is the macrophage. Based on external stimuli macrophages undergo classical M1 activation or alternative M2 activation. M2 macrophages are characterized by immunosuppressive and anti-inflammatory phenotype with lower IL12 and higher expression of IL10, TGF- β and arginase-1. In dextran-sodium-sulfate (DSS)-induced colitis mice model, infection with *Clonorchis sinensis* reduces inflammation by promoting IL10 production from macrophage M2, the non-inflammatory phenotype.

It is apparent that worms, which has co-evolved with the human host over millions of year has a symbiotic relationship. Its survival is depended on evasion of the host immune system through suppression of active immune response that could prevent autoimmunity and allergy as a beneficial spinoff which could be translated into therapeutic application.

CONCLUSION

Hygiene hypothesis has been validated through epidemiological, experimental and clinical observations. Its impact on several autoimmune diseases and allergic disorders has been critically evaluated with evidence emerging from the role played by helminths and its products in immune modulation. It acts on both the innate and adaptive immune system, down regulating

the inflammatory phenotype. Human clinical trials in the coming decades will provide exciting new parasite molecules for the treatment of autoimmune diseases.

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