TOURO COLLEGE & UNIVERSITY SYSTEM

The Science Journal of the Lander College of Arts and Sciences

Volume 8
Number 2 *Spring 2015*

1-1-2015

Understanding the Hygiene Hypothesis and its Mechanisms

Yaakov Tropper
Touro College

Understanding the Hygiene Hypothesis and its Mechanisms

Yaakov Tropper
Yaakov will graduate in June 2015 with a B.S. degree in Biology

Abstract
The hygiene hypothesis provides an explanation for the sharp increase in atopy over the past several decades by proposing that it is inversely related to the lack of infectious diseases in our society. Although atopy, as well as other hypersensitivity reactions, do have a genetic basis; studies clearly show that environmental and socioeconomic factors play a large role in determining which people will develop allergies. Proposed mechanisms leading to this disorder include an imbalance in the Th1/Th2 complex in the immune system, and a deficiency in Regulatory T cells which controls excessive T cell activity.

Introduction

The immune system is a continuously adapting organ system with many defense mechanisms in place to prevent infection. Thousands of microbes are constantly trying to find a way to infiltrate and take residence in the body and the components of the immune system together find a way to prevent infection from penetrating, or killing it once it does pierce the initial barrier.

Throughout history, the persistence of different infections has constantly evolved to reflect our continually changing ecosystem. As we learn to adapt to a specific disease, a new one will fill the void that was left. Yersinia pestis, the bacteria responsible for killing hundreds of millions of people throughout the 14th century is now almost completely eradicated (Haensch, et. al., 2010).

Many methods have been discovered which successfully wiped out several illnesses plaguing the globe for many years. Vaccinations, healthier diets, and personal hygiene have all had a positive effect in reducing the number of bacterial and viral infections throughout the past century. Mumps, measles, polio, and diphtheria are all examples of sicknesses that have practically been eliminated in most developed countries resulting in a healthier environment.

Despite the decreased incidence of microbial infections, many other disorders have quickly filled the void left by these infections. Crohn's disease, type 1 diabetes, and allergies have seen exponential growth over the past seventy years, indicating an increase in hypersensitivity reactions. This paper explores the recent increase in hypersensitivity reactions, specifically atopy, to see if it is a direct result of the decreased number of microbial infections.

Materials and Methods

Materials for this paper were mined using Touro College's library to access databases such as Proquest, Pubmed, and Ebsco. Additionally, some research was done using Google Scholar as well.

Discussion

The number of cases of autoimmune disorders skyrocketed over the past half century. Asthma, diabetes, and Crohn's disease are all examples of hypersensitivity disorders which have increased exponentially in recent years while bacterial infections have steadily decreased.

Bacterial infections and autoimmune disorders are believed to be inversely related. Evidence for this theory first appeared in 1989, in a paper written by David Strachan who conducted a study showing the relationship between the number of children born and the likelihood of receiving hay fever. Strachan found that children born into large families were less likely to contract the disease. He suggested the reason was that children born into large families were more susceptible to bacterial infections being transmitted by older siblings, preventing them from contracting allergies (Strachan, 1989). He did not provide an explanation for this correlation.

Strachan's hypothesis opened a door for many others who followed in his footsteps trying to explain the relationship between autoimmune disorders and bacterial infections. This theory later became known as the "hygiene hypothesis," and scientists today are still trying to find the mechanism which causes this correlation.

In order for scientists to properly study the mechanisms of infections leading to these disorders, it is important to understand what causes autoimmunity. Is a person's genetic makeup the primary factor causing the disease, or do socioeconomic factors determine if a person will develop the disorder?

Genetic Factors

It seems that there definitely are genetic factors that contribute to an individual's susceptibility to contract the diseases. Many human autoimmune diseases have a tendency for familial clustering. Familial clustering, represented by λs in Table 1 (Vyse & Todd, 1996), gives the ratio of clustering in families relative to the rest of the world. A λs of 15 means that a sibling is at 15 times greater risk of developing the disease than a member of the general population (Vyse & Todd, 1996). All of the autoimmune diseases listed contain a high rate of clustering, indicating that genes do play a role in autoimmune disorders. For type 1 diabetes, the MHC linked locus IDDM1 was the primary allele affecting the disorder, although other alleles played a smaller role as well.

Crohn's disease, another autoimmune disease affecting different areas of the gastrointestinal tract, is the result of many single nucleotide polymorphism (SNPs) throughout a person's genome (Mao & Jeonghwa, 2008).

Such evidence would disprove Strachan's hypothesis. If autoimmunity is solely a genetic disorder, it is impossible that environmental factors would be able to change an individual's chances of getting infected.

However, there are numerous studies that counter the notion that autoimmunity is affected exclusively through genetics. Firstly, evidence from observing distinct groups of individuals as they migrated from one region to another shows that the rate of developing diabetes is directly related to the location. A study regarding Jews emigrating from Yemen to Israel showed that the number of cases of type 1 diabetes sharply increased with the duration of their stay in Israel. In fact, the numbers were higher than any other ethnic group in Israel (Airaghi & Tedeschi, 2006).

Additionally, many studies have indicated that autoimmune disorders are not evenly distributed amongst the continents. Geographic data concerning Crohn's disease, multiple sclerosis, and type 1 diabetes demostrates that a north-south gradient exists (Bach, 2002). In the northern hemisphere, the incidence of autoimmune disease decreases towards the equator. One example of this is seen with the incidence of Crohn's disease in the US which shows a clear geographic pattern of occurrence. Data collected from hospital discharges suggests that Crohn's disease clearly occurs more often in northern parts of the country (Sonnenberg, et. al., 1991). Data exists showing similar results regarding type 1 diabetes, multiple sclerosis, and atopy as well (Bach, 2002). Genetic factors alone would not be enough to explain all of these phenomena.

Socioeconomic Status

Many studies have indicated that environmental factors, as well as socioeconomic status play an even greater role in determining one's chances in developing autoimmune disorders (du Prel, et. al., 2007). We already stated that autoimmunity is not evenly distributed among the continents. Developed countries usually have a higher rate of autoimmunity than developing ones (Corvalan, et. al., 2005). Even in developed countries, there are several factors that will determine an individual's chances of developing such a disorder. Socioeconomic factors can include a number of different variables. A study regarding type 1 diabetes in Germany went into greater detail to determine which factors will have a greater influence. The results indicated that some aspects have a greater effect than others. Income level, education, and other similar trends had a small effect while living space per person was a much larger influence in determining a child's risk of developing type 1 diabetes (du Prel, et. al., 2007). This study would support Strachan's theory that infections play a direct role on autoimmunity. Less living space would explain a child's susceptibility to get an infection which would inhibit his chances of developing type 1 diabetes. A similar study was done in Chile pertaining to asthma. The results supported the belief that overcrowding was associated with less asthma symptoms (Corvalan, et. al., 2005).

The general consensus is that although there are genetic variables in an individual that will determine if he is prone to develop an autoimmune disorder, these can be triggered based on socioeconomic conditions. A child raised in an environment that was exposed to infections was more prone to develop infectious diseases which in turn provided a sense of protection from developing autoimmune disorders.

Hypersensitivity

Although asthma and allergy are both subsets of hypersensitivity reactions, they are caused by very different mechanisms. Allergy is an immediate hypersensitivity reaction (Type 1 Hypersensitivity) caused by activation of Th2 cells (a subset of T cells) which release cytokines to produces IgE antibody. When atopic individuals encounter certain foods or pollen, they produce a dominant Th2 response which stimulates unchecked IgE production leading to an allergic response.

Autoimmunity is a delayed type (or Type 2) hypersensitivity reaction resulting from the immune system acting on self tissue. Although the direct cause for this disorder is still unknown, it is believed Th1 activity, normally responsible for activating this pathway, may be involved in causing this disorder (Lohoff, et. al., 1998).

Most of this paper focuses on allergy, because this is where most of the research on the hygiene hypothesis occurred, though some of the information may shed light on autoimmunity as well.

A simple explanation regarding why less infections are correlated with a higher rate of autoimmune disorders is that the more infections an individual undergoes in his lifetime causes a certain amount of pressure on the immune system, which has to fight off the antigen. Fewer infections means a delayed pressure on the immune system, which is free to mount inappropriate responses against self antigens as occurs in type 1 diabetes (Airaghi & Tedeschi, 2006).

This explanation would not fit into any of the mechanisms previously explained in triggering autoimmunity. Just because the immune system is not pressured to fight off antigens, this doesn't explain why it would fight self antigens. The hypothesis would be consistent if the immune system was weakened against foreign microbes but not for an entirely new disorder. Nothing in this

theory explains the inverse relationship between bacterial infections and autoimmunity.

Th1/Th2 balance

The immune system consists of T cells and B cells. T cells can be either CD8+, cytotoxic, which are responsible for killing viruses, or CD4+ helper T cells which assist in other immune functions. The CD4+ T cells can be either a subset of Th1 or Th2. Each subset helps the adaptive immune system in its own way.

Th1 cells are primarily instrumental in killing intracellular infections. Neutrophils are the immediate response to the body's defense against an attack. However, under some circumstances, these neutrophils are not successful in phagocytosis of the pathogen. Either the pathogen has evolved to evade the neutrophil, or the neutrophil has not been activated properly. These situations require help from Th1 activated T cells. These T cells recruit macrophages to kill the microbe through ROS (Reactive Oxygen Species). Although this is an effective way of destroying a pathogen, there are certain drawbacks. One issue is that the agent that kills the microbes may harm healthy tissue as well. Macrophages do not discriminate which species they attack so tissue damage is usually accompanied with hypersensitivity to an infection. One way of inhibiting this response is through Th2 activation.

The Th2 subset of T cells has an entirely different role than Th1. While Th1 is effective in activating macrophages, Th2 cells are primarily responsible for producing cytokines that signal antibodies such as IgE and activate eosinophils, which are responsible for killing Helminthic parasites (Abbas & Lichtman, 2009).

Th2 cells also secrete cytokines that activate the alternative macrophage pathway, which inhibits the classical macrophage pathway. When Th1 cells activate macrophages to kill a pathogen, they kill host tissue as well. Th2 cells are responsible for inhibiting this pathway, as well as producing cytokines to express mannose receptors to aid in tissue repair to the host.

A proper immune response to an infection requires Th1 cells to activate macrophages to fend off the infection and Th2 cells to slow down this response. Different organisms have different amounts of Th1 and Th2 which will directly affect how they respond to infections (Cohn, et. al., 2004).

An overabundance in Th2 response is what causes an allergic reaction. When a person is exposed to certain pathogens, namely Helminth parasites, Th2 cells secrete cytokines to bring IgE and mast cells to the area to kill the infection. Atopic individuals mount an especially strong immune response to these antigens causing an allergic response, known as type I hypersensitivity.

Th1 and Th2 develop from a common precursor termed Thp. Development into mature Th cell depends on the conditions present during maturation. Several cytokines such as IL2 and TGF-β have a tendency to cause Th2 proliferation. However in the presence of a normal amount of antigen in the host, antigen presenting cells produced IL-12 which caused Th1 proliferation (Lohoff, et. al., 1998).

Thus, a healthy balance of Th1 and Th2 is necessary for a proper immune response. Th1 is necessary to fend off pathogenic microbes at the site of infection while a proper Th2 response is necessary to prevent too much tissue from getting affected by the macrophages. Different amount of Th1 and Th2 in the body determines the outcome of an infection. An experiment with different strains of mice with different amounts of Th1 or Th2 helps determine the outcome of an infection. Leishmania major, an infection normally killed by activated macrophages, was placed in a strain of mice that had effective Th1 cells. The infection was successfully eradicated. However, when placed in a strain of mice that has a dominant Th2 presence, the mice succumbed to the infection (Elso, et. al., 2004). Mycobacterium leprae as well, if dominated by a Th2 response will lead to a much more severe form of leprosy.

This imbalance of Th1/Th2 might explain why less infection may cause more allergic hypersensitivity. Developed countries are certainly more hygienic and individuals are less prone to develop infections. The body has less of a need for Th1 cells and may suppress Th1 production. Once an imbalance is formed, Th2 secretes cytokines which further inhibits Th1 and cause an overabundance of Th2 production (Holt, 2000).

Regulatory T cells

In healthy individuals, regulatory T cells (Tregs) are responsible in suppressing dominant T cell activity. In response to infection, T cells often cause heavy responses that can cause serious harm to the individual if left unchecked. Activation of these Tregs, such as CD4+CD25+, were shown to suppress the Th2 response leading to a decrease of atopic responses in individuals exposed to pollen. Studies show that individuals who experience atopic symptoms do not express sufficient amounts of CD4+CD25+ (Ling, et. al., 2004). Additionally, lack of Tregs are also responsible for several disorders such as type 1 diabetes, multiple sclerosis, and inflammatory bowel disease.

Tregs utilize different mechanisms of control in response to different infections. In response to airway hypersensitivity reaction (AHR), Tregs secrete IL-10 along with TGF-β which controls the Th2 response. In NOD mice, evidence showed that Tregs were instrumental in preventing type 1 diabetes through activation of CTLA-4 (a cell surface molecule) together with TGF-β (Workman, et. al., 2009).

Alternatively, there are a number of factors that lead to healthy Treg development. FoxP3 is the transcription factor that develops immature T cells into regulatory cells. A mutation in this may lead to several autoimmune diseases such as diabetes, anemia, and eczema. Another cell necessary for healthy Treg development is IL-2. IL-2 is not secreted by Tregs but it is necessary for Treg survival. IL-2 is secreted by activated T cells and a healthy balance of is needed to maintain homeostasis. Effector T cells compete with Tregs for IL-2 so Tregs can upregulate CD25 production to suppress effector T cells, providing a healthy feedback mechanism between responder T cells and Tregs (Goleva, et. al., 2005).

The mechanism leading to this deficiency in Tregs is unclear. One theory suggests that the effector T cells in individuals with asthma may be toxic towards Tregs in these individuals (Thorburn & Hansbro, 2010).

Another theory is that Tregs may actually be induced by pathogenic infections as indicated in Table 2 (Thorburn & Hansbro, 2010). Antigen presentation by the lungs was shown to promote TGF-β dependent induction of Foxp3 expression. Lack of infection may lead to an uncontrolled immune response. Several infections are known to have a direct link to regulatory cells so by inhibiting these cells; it would lead to an increase in allergic response.

One particular study tried to find if there is a direct correlation between asthmatic infection and H. pylori- a common bacteria found in the gut. Experimentally induced asthma in mice had fewer symptoms when the mice were previously infected with H. pylori. The study also showed that these mice had significantly higher numbers of Tregs leading to the belief that Tregs is one of the underlying mechanisms controlling an asthmatic response (Arnold, et. al., 2011).

Microbiota Theory

Although this study definitely supports the theory that Tregs are one of the most influential factors in developing asthma, it also supports a different belief entirely in what causes the hygiene hypothesis.

In an essay concerning the diminishing numbers of microbiota in the body, Blaser and Falkow postulated that the main cause in the increased numbers of autoimmune disorders is related to the number of microbiota in body, rather than the number of pathogenic infections a person encounters throughout life. For the past several centuries, humans have adapted to allow a diverse subset of microbial organisms to live inside the mucosal surfaces of the intestines and lungs, forming a symbiotic relationship between host and microbe. Humans provide a home for these microbes while they are helpful in digestion and metabolism. A change in human behavior may have a direct effect on how people develop certain microbiota as shown in Table 3. The changes in the conditions listed have had a direct effect on many of the known microbes that reside in the human body. Although reduced colonization of H. pylori from the gastrointestinal tract is beneficial in the sense that reduces susceptibility to gastric cancer, it is also shown to contribute to many metabolic disorder as well as childhood asthma. Blaser and Falkow (2009) maintain that the recent epidemic of autoimmune disorders is not due to the lack of pathogenic infection as much as it is due to the human microbiome as a whole (Blaser & Falkow, 2009). Other research supports this premise by saying the hygiene hypothesis may not a product of less exposure to pathogenic infections itself, as much as it is caused by lack of exposure to all microorganisms (Bloomfield, et. al., 2006)

Conclusion

Despite all the evidence supporting the hygiene hypothesis, there still is a fierce debate whether less hygiene will in fact cause a downward trend in autoimmune diseases. The direct mechanism remains elusive, and without definitive evidence demonstrating what causes the rise in allergic diseases, the microbial exposure hypothesis may be just as plausible in explaining the rise in atopy. And considering the many benefits our modern ecosystem provides for us on a daily basis, the pros of less microbial exposure far outweigh the cons. Clean drinking water and Cesarean sections are just a few examples that have saved many lives over the past few decades. So although decreasing the number of allergy cases is a priority, the current evidence should not support exposing oneself to additional microorganism than those already prevalent in society.

Table 3

Change	Consequence
Clean water	Reduced faceal transmission
Increase in Caesarean section	Reduced vaginal transmission
Increase use of pre-term antibiotics	Reduced vaginal transmission
Reduced breastfeeding	Reduced cutaneous transmission and a changed immunological environment
Smaller family size	Reduced early life transmission
Widespread antibiotic use	Selection for a changing composition
Increased bathing, showering and use of antibacterial soaps	Selection for a changing composition
Increased use of mercury-amalgam dental filling	Selection for a changing composition

Changes in human ecology that might affect microbiota composition

References

Abbas, A. K., & Lichtman, A. H. (2009). Basic Immunology- Functions and Disorders of the Immune System (3rd Edition ed.). Philadelphia, PA, USA: Saunders Elsevier.

Airaghi, L., & Tedeschi, A. (2006). Negative Association between occurrence of type 1 diabetes and tuberculosis incidence at population level. Acta Diabetil.

Arnold, I. C., Dehzad, N., Reuter, S., Martin, H., Becher, B., Taube, C., & Muller, A. (2011). Helicobacter pylori infection prevents allergic asthma in mouse models through the inductio of regulatory T cells. Journal of Clinical Investigation, 3088-3093.

Bach, J.-F. (2002). The Effect of Infections on Susceptibility to Autoimmune and Allergic Diseases. New England Journal of Medicine, 911-920.

Blaser, M. J., & Falkow, S. (2009). What are the consequences of the disappearing human microbiota? Nature Reviews. Micorbiology, 887-894.

Bloomfield, S., Stanwell-Smith, R., Crevel, R., & Pickup, J. (2006). Too clean, or not too clean:the Hygiene Hypotheses and home hygiene. Clinical and Experimental Allergy, 402-425.

Cohn, L., Elias, J. A., & Chupp, G. L. (2004). ASTHMA: Mechanisms of Disease Persistence and Progression. Annual Review of Immunology, 789-815.

Corvalan, C., Amigo, H., Bustos, P., & Rona, R. J. (2005). Socioeconomic Risk Factors for Asthma in Chilean Young Adults. American Journal of Public Health, 1375-81.

du Prel, J., Icks, A., Grabert, M., Holl, G. G., & Rosenbauer, J. (2007). Socioeconomic conditions and tyoe 1 diabetesin childhood in North Rhine-Westphalia, Germany. Diabetology, 720-728.

Elso, C., Roberts, L., GK, S., RJ, T., Baldwin, T., Foote, S., & Hnadman, E. (2004). Leishmaniasis host response loci (lmr1-3) modify disease severity through a Th1/Th2-independent pathway. Genes and Immunity, 93-100.

Goleva, E., Cardona, I. D., Ou, L.-S., & Leung, D.Y. (2005). Factors that regulate naturally occuring T cell regulatory cell-mediated suppression. Journal of Allergy and Clinical Immunology, 1094-1100.

Haensch, S., Bianucci, R., Signoli, M., Rajerison, M., Schultz, M., & Kacki, S. (2010, Oct 7th). Distinct Clones of Yersinia pestis Caused the Black Death. PLOS Pathogens.

Holt, P. G. (2000). Parasites, atopy, and the hygiene hypothesis: resolution of a parpadox? The Lancet, 1699.

Ling, E. M., Trevor, S., Nguyen, X. D., & Carol, P. (2004). RElation of CD4+CD25+ regularory T-cell suppression of allergen-driven T-cell activation ot atopic status ad expression of allergic disease. The Lancet, 608-15.

Lohoff, M., Gessner, A., Bogdan, C., & Gollinghoff, M. (1998). The Th1/Th2 Paradigm and Experimental Murine Leishmaniasis . International Archivesof Allergy and Immunology, 191-202.

Mao, W., & Jeonghwa, L. (2008, May). A Combinatorial Analysis of Genetic Data for Crohn's Disease. Journal of Biomedical Science and Engineering, 52-58.

Sonnenberg, A., McCarty, D., & Jacobsen, S. (1991). Geographic variation of inflammatory bowel disease within the United States. Gastroenterology.

Strachan, D. (1989). Hay fever, hygiene, and household size. BMJ, 1259-1260.

Thorburn, A. N., & Hansbro, P. M. (2010). Harnessing Regualtory T cells to Suppress Asthma: From Potential to Therapy. American Journa of Respiratory Cell and Molecular Biology, 511-9.

Vyse, T. J., & Todd, J. A. (1996). Genetic Analysis of Autoimmune Disease. 311-318.

Workman, C. J., Szymczak-Workman, A. L., Collison, L. W., & Pillai, M. R. (2009). The development and function of regulatory T cells. Cellular and Molecullar Life Science, 2603-2622.