E&EB 235: Evolution and Medicine
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By submitting this essay, I attest that it is my own work, completed in accordance with
University regulations. —Stacy Scheuneman

<u>The Hygiene Hypothesis and the Increase of Cancer in the 20th Century</u>

by Stacy Scheuneman

Abstract

Cancer is a leading cause of death in the United States and other developed countries, spiking in incidence in the 20th century. Along with other explanations, the hygiene hypothesis should be considered. The hygiene hypothesis posits that the rise in autoimmune and atopic diseases in the twentieth century arises from the decrease in exposure to microbial environmental factors that co-evolved with our immune system. In order to establish the significance of the relationship between cancer incidence and pathogen exposure, correlations between cancer incidence and per capita GDP, life expectancy, tobacco consumption, and fertility were calculated. A significant strong positive association between cancer incidence and per capita GDP was found, while those between cancer incidence and life expectancy, tobacco consumption, and fertility were weak and not significant. In light of these results, a connection between the hygiene hypothesis and inflammation as a cause of cancer was drawn, and treatment with pathogens, specifically virotherapy, is discussed. Finally, further research on the hygiene hypothesis in relation to cancer is recommended.

Introduction

Cancer is the second leading cause of death in the United States, preceded only by heart disease (1). Cancer is a category of diseases that is characterized by uncontrolled, abnormal cell division that arises from a sequence of mutations (2). The most common treatments for cancer are chemotherapy, radiation therapy, and surgery (3). Unfortunately, chemotherapy and radiation therapy tend to give rise to more potent strains of cancer by selecting for resistance and

eliminating competition between resistant and sensitive strands (4). Therefore, novel approaches to preventing and treating these diseases and utilizing evolutionary theory are important steps that need to be taken in cancer treatment policy.

There has been an increase in all cancers over the course of the twentieth century. Three evolutionary explanations for cancer in humans are longer life span, which leads to overall increase in cell divisions within an organism; exposure to new carcinogens, which increases the mutation rate; and delayed and decreased reproduction in women, which increases the number of menstrual cycles per lifetime and therefore the number of cell divisions in some reproductive tissues (5). In this paper, I would like to examine another less common explanation: the hygiene hypothesis.

Worldwide, there is a higher incidence of cancer in developed countries, while there are lower rates in less developed countries (10). This relationship can be seen at a glance in Figure 1, a map depicting the rates of incidence of all cancers in men (a similar trend is also apparent in women, which was not included in this analysis to save from redundancy). Lighter areas have low incidences and darker areas have high incidences (10).Here, I offer an additional explanation – cancer is more prevalent in countries with higher per capita gross domestic products, and as a result, with lower exposure to environmental microbes.
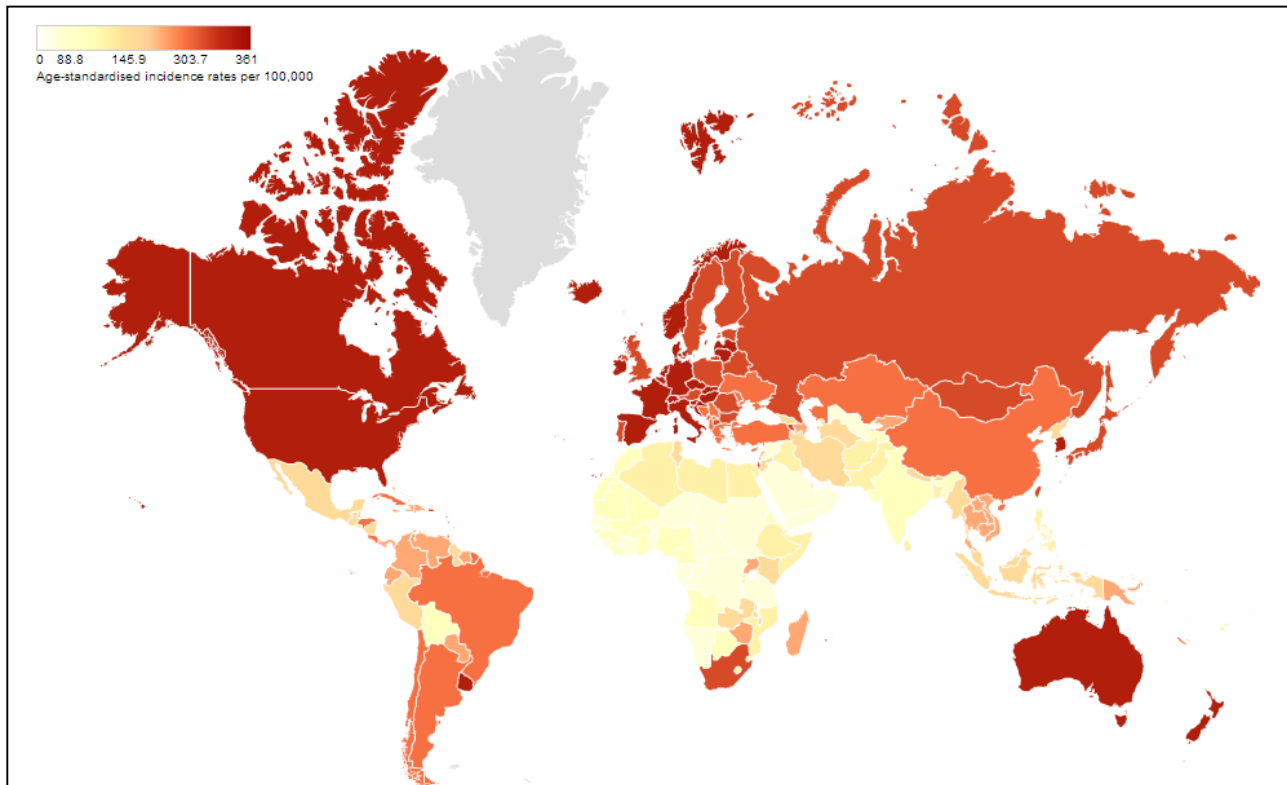
Figure 1. Cancer incidence in men, cases per 100,000 (10)
This map shows cancer incidence by country. At a glance, it can be seen that the developed countries of North America and Western Europe have the highest rates of cancer (darkest colors are highest incidence) while South America, South Asia, and Africa have the lowest rates.

The Hygiene Hypothesis, also called the Old-Friends Hypothesis, is a hypothesis that accounts for the rise in autoimmune diseases in the twentieth century with the decrease of exposure to microbial environmental factors that co-evolved with our immune system (6). Several recent studies have demonstrated a connection between autoimmune diseases and parasitic worms, where parasite infections have decreased the intensity of symptoms of patients with multiple sclerosis (7, 8, 9), but few have looked at cancer in light of this hypothesis. In this paper, I will demonstrate why the hygiene hypothesis should be looked at more closely in connection to cancer. In order to do so, I will first look at the increasing incidences of cancer as time goes by and the relationship between incidences of cancer and the development status of countries. Then, I will look at the literature on the relationship between hygiene and

inflammation and the relationship between inflammation and cancer. Finally, I will look at the research that has been done treating cancer with viruses.

Materials and Methods

In order to support the assertion that there is a relationship between a country's GDP and its cancer incidence, I used data from the World Health Organization GLOBOCAN Project (cancer incidence rates per 100,000 people), the Central Intelligence Agency (per capita GDP), and the Organisation for Economic Co-operation and Development (life expectancy at birth, percentage of people over fifteen years that are daily smokers, and fertility rates) to calculate correlations between cancer incidence and per capita GDP, life expectancy, tobacco consumption, and fertility. Cancer incidence data from GLOBOCAN is derived from population-based cancer registries, which, in many developing countries, takes into account mainly major cities; the incidence numbers are for the year 2008 (11).

All calculations were performed in the statistical program Minitab. Commands used were MTB > corr 'variable1' 'variable2', with "variable1" and "variable2" corresponding to one of the two variables in each analysis. Graphs were also produced using Minitab, using the commands Graph > Scatterplot > Simple. GDP was used as an indicator of microbial exposure in order to enable utilization of a larger sample, since there is more consistent data on national GDP than there is for say, death from microbial diseases, especially for developing countries. In general, countries with lower GDPs spend less money on public health and have fewer resources like clean water supply and sanitation (33).

Results

Using the cancer incidence data and the per capita GDPs of the 100 most populous countries (12, 13), the correlation between cancer cases per 100,000 people and per capita GDP

was calculated. The Pearson correlation of per capita GDP and cancer cases per 100,000 people was found to be 0.857, a strong positive association, with a p-value of <0.001, which assures us that the correlation in the data does not support the null hypothesis that there is no association between cancer incidence and per capita GDP. A scatterplot of this data is included below.

However, per capita GDP is not the only factor unique to the people of a country. The other factors contributing to a rise in cancer incidence, as mentioned earlier, include increased longevity, exposure to new carcinogens, and delayed and decreased reproduction. To test whether these factors shared the same association with cancer incidence, I calculated correlations between cancer incidence and life expectancy, tobacco consumption, and fertility as well.

Using numbers from the Organisation for Economic Co-operation and Development, I calculated the following correlations. The Pearson correlation of life expectancy at birth and cancer cases per 100,000 people is 0.286, a weak positive correlation, with a p-value of 0.106, meaning that it is possible that this correlation is due to chance.

Furthermore, while longer life-spans can explain the increase in late-life cancers, they cannot explain the increase in childhood cancers, which would not necessarily result from degeneration. An increase not only in cancer in adults has occurred over the course of the 20th century, but also in childhood cancers. The incidence rate for all types of cancers in children under the age of fifteen has increased, from 11.5 to 14.8 cases per 100,000 children from 1975 to 2004 (14) – a 22% increase in just under three decades. Additionally, there is a moderate positive correlation between childhood cancer incidence rates and per capita gross national income, seen in the plot below (15).
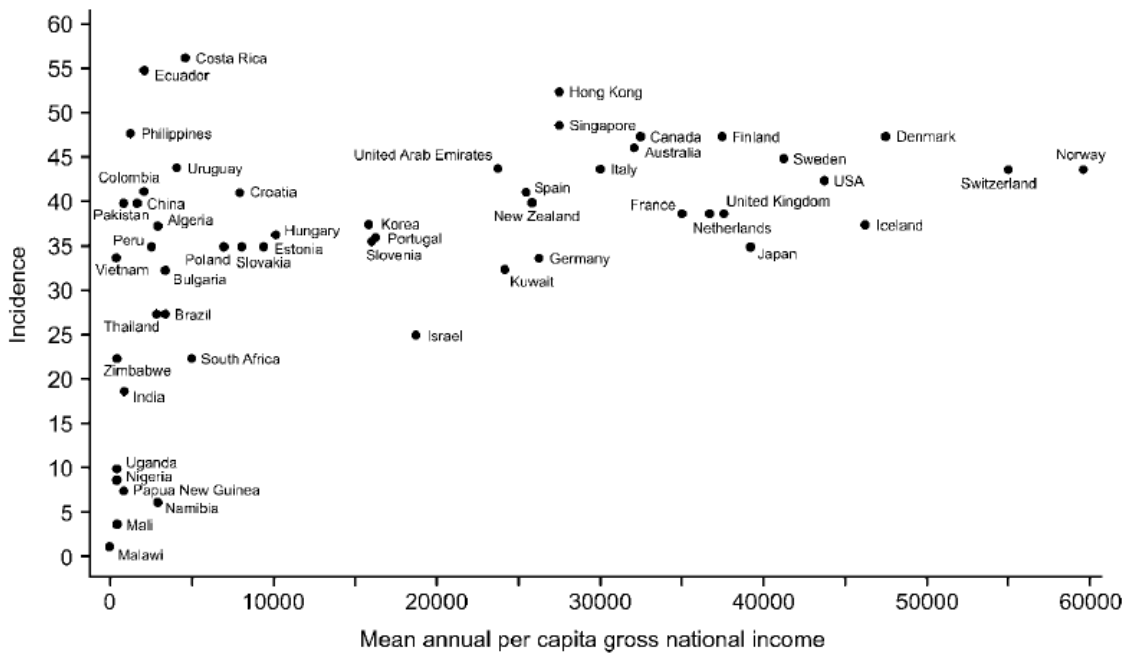
Incidence (y-axis): 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60

Mean annual per capita gross national income (x-axis): 0, 10000, 20000, 30000, 40000, 50000, 60000

Data labels: Costa Rica, Ecuador, Hong Kong, Singapore, Canada, Finland, Denmark, Philippines, Australia, Sweden, Norway, Uruguay, United Arab Emirates, Italy, USA, Switzerland, Colombia, Croatia, Spain, France, United Kingdom, China, Algeria, New Zealand, Netherlands, Iceland, Pakistan, Korea, Portugal, Hungary, Japan, Peru, Estonia, Germany, Vietnam, Poland Slovakia, Slovenia, Bulgaria, Kuwait, Brazil, Thailand, Israel, Zimbabwe, South Africa, India, Uganda, Nigeria, Papua New Guinea, Namibia, Mali, Malawi

Figure 2. Scatterplot of childhood cancer incidence vs. mean annual per capita gross national income (15)

The graph shows a positive association between mean annual per capita gross national income and childhood cancer incidence.

In regards to tobacco consumption, the Pearson correlation of the percentage of the population fifteen years and older that are daily smokers and cancer cases per 100,000 is -0.137, a weak negative correlation, with a p-value of 0.448, meaning that there is almost a 50% probability that this correlation is due to chance. The Pearson correlation of fertility rate and cancer cases per 100,000 is 0.034, a weak positive correlation, with a p-value of 0.869 – almost a 90% probability of resulting from chance.

These results may be exaggerated; for the per capita GDP-cancer incidence correlation, the data set of 100 countries was much larger than those for the latter three, which were 33, 33, and 26, respectively. All of the countries included in these sets were located in Europe, North America, South America, Asia, and Oceania – excluding many of the lowest income nations in Africa and many of the lower income nations in South America and Asia. When only the 33

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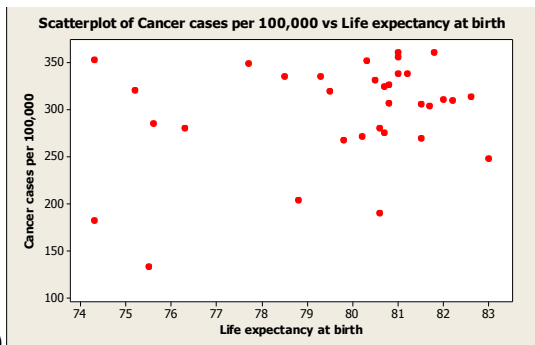**Scatterplot of Cancer Cases per 100,000 people vs Per Capita GDP**
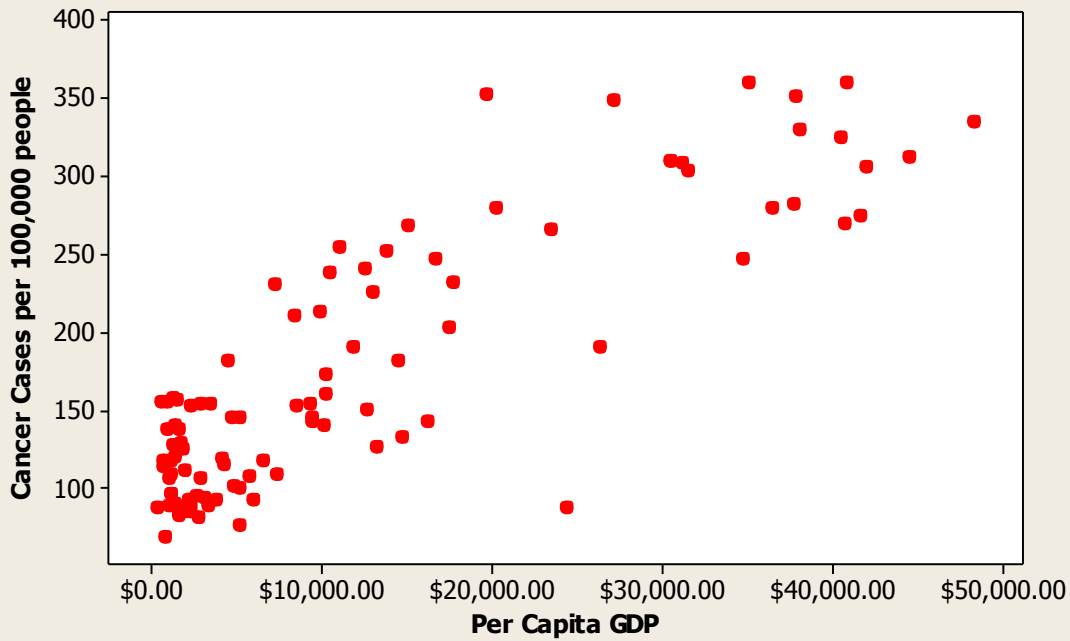
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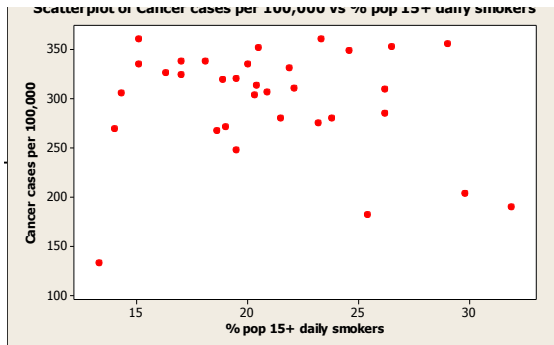presumably exposure

However, for all of

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Figure 3. Scatterplot of cancer cases per 100,000 people vs. per capita GDP using 100 countries

the more wealth a country has, the better its ability to keep track of cancer.

strong positive association can be seen on this graph.
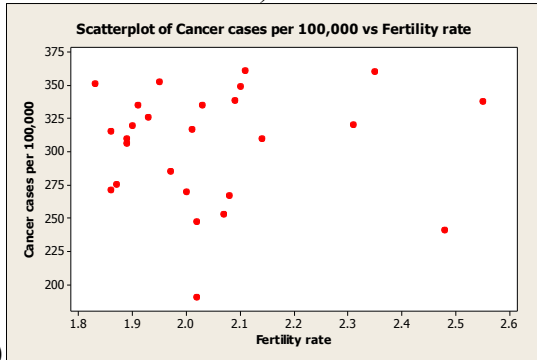
Tables and Graphs
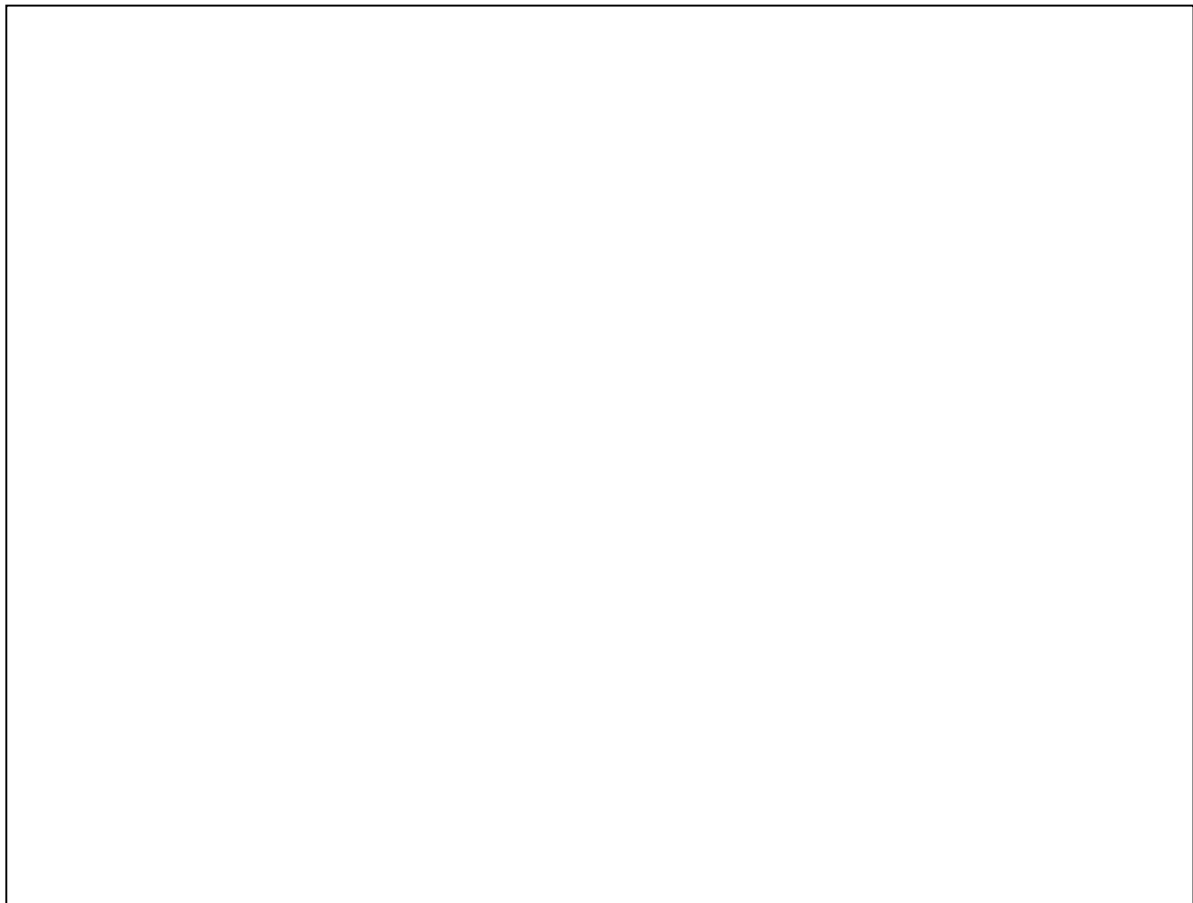
Figure 3. a) Scatterplot of cancer cases per 100,000 people vs. life expectancy at birth. A weak positive association can be seen in the plot. b) Scatterplot of cancer cases per 100,000 people vs. percentage of the population, aged fifteen and older, who are daily smokers. A very weak negative association can be seen in the plot. c) Scatterplot of cancer cases per 100,000 people vs. fertility rate. A very weak positive association can be seen in the plot.

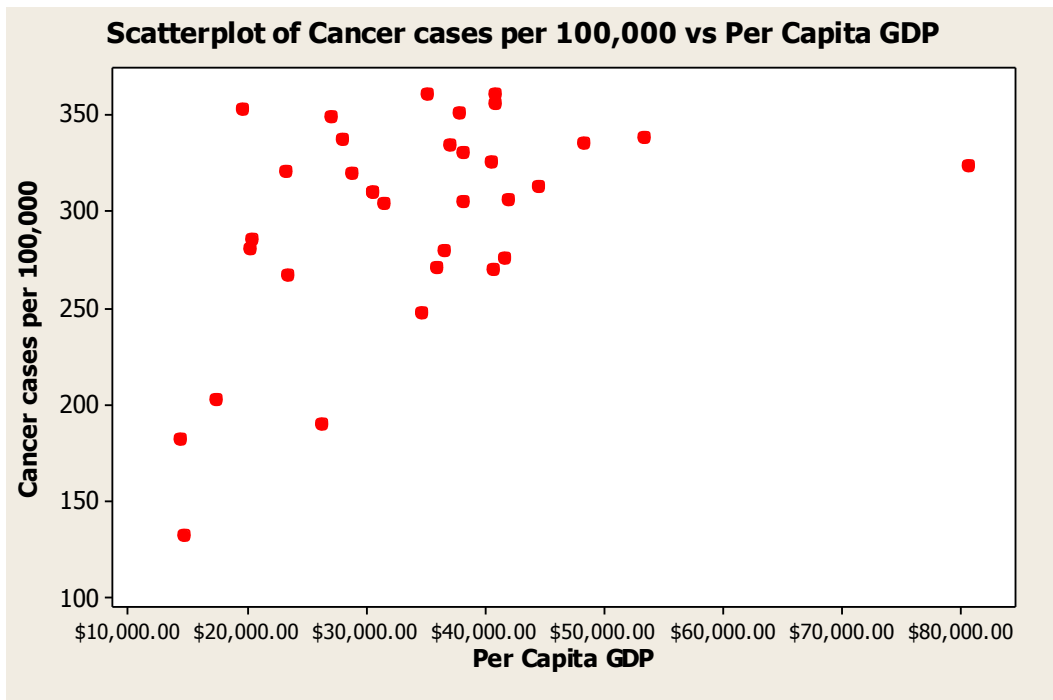Scatterplot of Cancer cases per 100,000 vs Per Capita GDP

Figure 4. Scatterplot of cancer cases per 100,000 vs. per capita GDP using 33 countries. A strong positive association can be seen in the plot, although a weaker one than was observed in Figure 3.

Discussion

The Hygiene Hypothesis, Inflammatory Immune Response, and Cancer

The hygiene hypothesis arose as an explanation for atopic and autoimmune diseases, and its application can be seen through a similar lens. Starting in 1989, when David Strachan first observed the correlation between family size and incidence of hay fever (16), the "hygiene" or "old friends" hypothesis has been increasingly examined as a possible explanation for the rise in auto-immune diseases, allergies, and asthma. This hypothesis suggests that our change in environment from hunter-gatherer to post-industrial conditions and our consequent reduced exposure to microorganisms has caused a disparity in our necessary immune reaction to our realized immune reaction, leading to an increase in inflammatory disorders (17). A more general

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explanation is that reduced exposure to microorganisms early in life leads not only to diagnosable atopic and autoimmune diseases, but also to increased inflammation in general, later in life.

Chronic inflammation has been established as a cause of many cancers (34, 35, 36). For example, ulcerative colonitis is a risk for colorectal cancer, which can be reduced by treatment with the anti-inflammatory 5-ASA medication (18). The role of Epstein-Barr virus in mononucleosis and Hodgkin's lymphoma is another example (19). EBV is a herpesvirus that infects over 90% of the world's population, usually during childhood. If EBV is contracted during childhood, symptoms are generally mild; however when EBV is contracted during or after adolescence, approximately half of infections develop into infectious mononucleosis, which increases the risk of developing Hodgkin's lymphoma threefold (19). A correlation between attendance in nursery or day school prior to kindergarten and lower risk of Hodgkin's lymphoma was observed (19). The significance of attendance in nursery or day school is that, by being in contact with many other children at a young age, children that attended had a higher exposure to pathogens than those who did not. Other pathogens and associated cancers include Hepatitis virus (which infects the liver) and liver cancer (20), the parasite Schistosoma haematobium (which infects the bladder) and bladder cancer (20), and the bacteria Helicobacter pylori (which causes stomach ulcers) and stomach cancer (20).

The development of vaccines to prevent cancer demonstrates further proof that the hygiene hypothesis has significant application to cancer. By vaccinating against cancer-associated viruses, major inflammation is prevented. Kuper et al. describes three mechanisms through which infectious agents can lead to cancer. The first is transforming cells by inserting oncogenes into the host genome, inhibiting tumor suppression, or stimulating cell division (21).

The second is suppressing the immune system, which results in reduced immunosurveillance (21). The third is the induction of chronic infection and inflammation, which results in DNA damage and increased division for repair (21). Without vaccination, an encounter with a virus would lead to a more intense infection, with greater inflammation as a result. On the other hand, vaccinating with an attenuated virus would elicit a milder immune response than a full-on infection by the virus occurs. In the case of many viruses, early exposure has a similar effect, such as infectious mononucleosis-inducing EBV previously mentioned (19). In the cases of vaccination and early exposure, evidence points to there being an advantage to being exposed to some pathogens early in life. Living in an environment with earlier and more exposure to environmental pathogens could possibly result in lower inflammation and therefore lower incidences of cancer.

Early Treatment of Cancer with Virotherapy

Studies have shown that removing infection from patients with multiple sclerosis results in an aggravation of symptoms (7, 8). The basis of these studies' hypotheses is the association between higher incidence of atopic diseases and lower exposure to microbes in the environment. As we have seen, a similar association can be observed between incidence of cancer and lower microbe exposure. However, there has not been significant research on the effect of parasite infection in cancer patients. However, there has been a large amount of work done in cancer virotherapy. Many of the studies show a positive reaction of patient health to virotherapy, although the mechanisms are still largely unknown and disputed.

Research on the treatment of cancer using oncolytic viruses was conducted as early as 1950, and appears to be a promising field. In a case study in 1950, George Pack first observed that viruses might be used to treat cancer when a woman with malignant melanoma who was

having consistent removal of melanotic nodules when she was bitten by a dog and had to have fourteen injections of rabies vaccination. Only two more nodules were removed five years later, and the woman had no more recurrence afterwards (22). This was followed up by a clinical trial, in which twelve patients were treated daily with inoculated rabies vaccines for 20 days. However, the results of this trial were largely unsuccessful; ten of the twelve patients had no change in tumor size, while two showed some limited and temporary improvement (22). It is not specified whether these patients had ever been exposed to rabies virus or vaccine prior to the study, which may be an important factor in success of a virotherapy, since the immune response to the vaccine presumably plays a role. In 1952, Southam and Moore showed that the Egypt 101 virus localized only in tumors and not healthy tissue, but the virus demonstrated no therapeutic value (23). In 1956, Smith et al. used APC viruses grown in HeLa cell and KB cell cultures and treated 30 patients with cervical epidermoid carcinoma. The treatment resulted in necrosis of the tumor and cavity formations within the tumors, but after treatment ended the tumors continued to grow. Additionally, effects were seen mainly in patients who had had little or no antibodies against the virus (24), supporting the importance of exposure to the virus to the efficacy of the treatment. These early experiments suggest that virotherapy could possibly work in naïve patients, but fail when the patient is able to produce a rapid immune response to the virus.

In one early trial in 1974, active mumps virus was used to treat various tumors. When treatment was interrupted, the tumor grew again (25). In 37 out of 90 patients, the tumors decreased in size to less than half of the initial size of the tumor; in 42 of the 90 patients, the tumor showed a tendency of growth suppression or retreat; and in the remaining 11 patients there were no significant effects (25). Several cancers were treated in this study, implying that the effects are perhaps the result of a more general reaction. Also, few side-effects were observed,
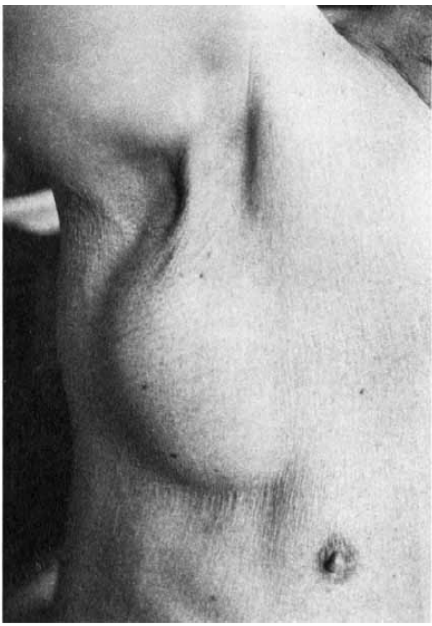
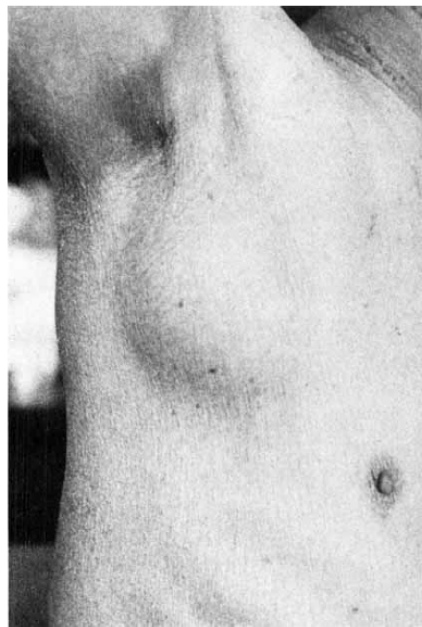Fig. 27. Malignant lymphoma of the right armpit (Case 7).

b)

Fig. 29. Reduced tumor (Case 7) on the 10th day after MVT.

had locali... ...fecting healthy tissue. In ...Figure 5, ...study, the tumor after ...ced compa...

...gure 5. Tumor before (a) and after (b) treatment with active mumps virus (25). The ...mor is visibly greatly reduced in size

In a 1976 study, treatment with bovine enterovirus was administered on the sixth day to mice with Ehrlich ascites tumors and Sarcoma-1 ascites tumors (ascites is peritoneal cavity fluid), which were maintained and then transferred to the thighs of the Swiss-Webster mice used in the study (26). For the first six days, weight increased for all mice (26). Mice that were not treated and mice that were given a UV-treated form of the virus continued to gain weight after day six, while the average weight gain of mice that were given active virus decreased on day six (26). The conclusions of this study are based on the assumption that weight gain is associated with tumor size increase, although weight gain can also be a sign of health. However, the steady increase in average weight gain for the first six days of unmitigated tumor growth supports this

assumption and the conclusion. Another important observation in this study was the lack of necrosis of muscle tissue in mice with solid tumors compared to control mice treated with the live virus, which did experience muscle tissue necrosis (26). The study doesn't address the problem of what would happen after the tumor has disappeared, however. This localization of the effects of the virus in the tumor may be evidence for some evolutionary natural interaction between cancerous tumors and certain viruses.

Recent Treatment of Cancer with Virotherapy

In 1994, a study of the treatment of athymic mice (with HT1OSO fibrosarcoma xenografts) with Newcastle disease virus yielded complete regression of tumors in 8 out of 10 mice, and tumors did not grow back during the one year follow-up period. In the control mice, which were treated with phosphate-buffered saline (PBS), all 9 mice showed tumor growth. This was repeated in athymic mice with Th15145 synovial sarcoma xenografts. In the 9 mice treated with Newcastle disease virus, over 80% regression occurred in tumors. The mice treated with PBS once again, showed tumor growth (27).

More recently, Peng et al. performed studies treating human epithelial ovarian xenographs (28) and myeloma xenographs (29) in mice using an engineered measles virus. In mice with the ovarian tumors, the engineered MV-Edm virus caused complete regression of 80% of the tumors and enhanced the survival of the mice by more than 50 days. In the mice with the myeloma xenographs, the virus caused tumor regression and also showed selective replicativity, replicating in the myeloma cell lines but not in the normal cell lines.

Another important factor in the success of a treatment seems to be the use of a live virus versus use of an attenuated virus or vaccine. When a live virus is used, more positive effects are observed, while when an attenuated virus or vaccine is used, little or weak anti-tumor effects

were observed. Although several of the studies discussed have shown evidence that the viruses used show tumor specificity, the issue of what would happen to the virus after the tumor is reduced sufficiently; would the virus then attack healthy tissue? It has not been studied whether normal immune response to the virus continues to develop while the virus is concentrated in the tumor and would therefore be cleared once it enters healthy tissue, or if infection would persist.

These studies show support for the efficacy of treating cancer with viruses, and perhaps other microbes such as parasites. The mechanism for these treatments is still unknown. It was suggested that a mutation of p53, a tumor suppressor gene which is inactive in many human cancers, could be the target for some of these viruses (30). ONYX-051, the first genetically engineered replication competent virus to have specific antitumor effects, was more effective in p53 mutant tumors than it was in p53 wild-type tumors (30). However, contradicting studies refute this. Oshea et al demonstrated that late viral RNA export, and not p53 degradation, results in the tumor specific replication of ONYX-015 (31). Rothmann et al reported that ONYX-015 replicates independently of p53 status and showed that the virus was able to kill non-tumor cells (32), leaving the molecular basis for replication differences in different cell types undetermined.

This, along with the 1974 study by Asada et al using active mumps virus to treat various tumor types, suggests that the explanation for viral-induced tumor necrosis may involve an immune reaction, and does not solely depend on the viruses' regulation of gene expression.

Conclusion

There is strong preliminary evidence, including the demonstrated positive association between per capita GDP and cancer incidence, as well as the relationship between late exposure to pathogens, inflammation, and oncogenesis, that the hygiene hypothesis can be applied to not only atopic diseases, but to cancer as well. Some thought has been given to this in the academic

field, but it remains a highly overlooked explanation. Although research on virotherapy does not aim to prove a link between the Hygiene Hypothesis and cancer, it makes a strong case for it regardless.

Cancer prevention depends on the control of chronic inflammation early on in cancer development, much before diagnosis. Vaccinations are one means by which we can do this – the little inflammation caused by a vaccine would later save the body from a much more aggressive inflammation caused by a live virus. Developed countries enjoy many benefits from decreased early exposure to environmental pathogens, such as lower mortality rates and lower infant mortality rates from infectious diseases, in exchange for much higher cancer incidences and deaths. Ideally, a safe way to produce the same effects of early exposure to pathogens could be developed so that cancer, as well as atopic and autoimmune diseases, could be prevented. Further research needs to be done in order to reinforce these ideas and unveil further directions that should be followed.

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