EBV and Cellular Immune Deficiencies

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EBV serology Testing Observations

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Cellular Immune Deficiencies as the Underlying Cause of Elevated Epstein-Barr Virus Antibody Titers in EBV-Associated Illnesses.

I became interested in EBV while working in the lab where I am employed. EBV has been associated with many illnesses including chronic fatigue syndrome (which has been pretty much disproved at this time). I feel that the presence of elevated levels of EBV antibodies may be used as markers for other illnesses in individuals that have cellular immune deficiencies.

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Cellular Immune Deficiencies as the Underlying Cause of Elevated Epstein-Barr Virus (EBV) Antibody Titers in EBV-Associated Illnesses.

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The text presented here is in a summary form.

Abstract

Epstein-Barr virus (EBV) is indigenous to all environments, infecting most of the human population. Its presence is easily demonstrated by serological tests. Primary infection with EBV results in infectious mononucleosis. However, increased levels of IgG antibodies to viral antigens are

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Rheumatoid Arthritis
Nasopharyngeal Carcinoma
Burkitt's Lymphoma
Hodgkin's Disease
Sjogren's Syndrome
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associated with several chronic illnesses, such as carcinomas and autoimmune disorders. This thesis proposes a test to distinguish whether EBV infection is a cause of these illnesses, or simply an associated marker. It is proposed that cellular immune deficiencies, involving decreased cytotoxic/suppressor T cell and/or natural killer cell function, cause these chronic illnesses, and that elevated EBV antibodies reflect the underlying cellular immune deficiencies.

List of Abbreviations

BL  Burkitt's lymphoma
CFS  chronic fatigue syndrome
CD  cluster of differentiation- the designation given to antigens on lymphocytes detected with monoclonal antibodies
CD2  the antigen which appears on all T cells
CD4  the antigen which appears on all helper/inducer T cells
CD8  the antigen which appears on all cytotoxic/suppressor T cells
CD16  the antigen which appears on all natural killer cells
CD20  the antigen which appears on all B cells
EA  Epstein-Barr virus early antigen
EBNA  Epstein-Barr nuclear antigen
EBV  Epstein-Barr virus
EBV-VCA  Epstein-Barr viral capsid antigen
NK  natural killer cells
NPC  nasopharyngeal carcinoma
RA  rheumatoid arthritis

Background

Epstein-Barr virus (EBV) is a ubiquitous virus which infects 90% of the human population by adulthood. It is a DNA virus and, although immunologically unrelated, it is classified as a member of the herpesviruses. Primary infections in third world countries usually occur in early childhood and are subclinical. In more developed countries primary infections usually occur at a later age and may present themselves as acute infectious mononucleosis.

It is now believed that at least 90% of EBV infections are transmitted orally through mouth to mouth exchange of infected saliva. The epithelial cells of the oropharynx serve as a reservoir of the virus and periodically shed virus into the saliva. Many tissues and organs of the body may become infected with EBV, the principal ones being the epithelial cells of the oropharynx and nasopharynx, and the B lymphocytes of the immune system. It is the initial infection of the B cells and the immune system's response to this infection which leads to the symptoms seen in infectious mononucleosis.

Following acute infection the virus becomes latent and, as with other herpesviruses, may be reactivated at a later time. This reactivation of infection in the oropharynx and shedding of
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virus into the saliva is believed to allow passage of virus from one individual to another. Latent infection has been well documented in B lymphocytes. Virus infected B cells remain in the circulatory system for months, somehow avoiding both cellular and humoral immune detection. As a result of acute infection memory T and B cells are produced which will respond to EBV if stimulated by the virus. The reason for the lack of immune response to the infected B lymphocytes is not clearly understood. It is known that once B cells are infected, viral replication is held in check. Infected B cells are also prevented from further differentiation and remain in an intermediate (proliferative) stage without progressing to antibody-producing plasma cells. It is, however, believed that infected B cells are responsible for spreading EBV infection to various tissues and organs of the body.

Epstein-Barr virus infects B cells by attaching to the C3d receptor on the cell surface of the lymphocyte. This receptor is also designated as the CD21 antigenic determinant. The virus may also infect any cell type which has this same or similar receptor located on its cell surface, such as pharyngeal epithelial cells (Allday & Crawford, 1988; Grierson & Purtillo, 1988; Thorley-Lawson, 1988).

When primary EBV infection occurs as infectious mononucleosis, the transient "virus-driven B-cell proliferation" is "countered by an unusually exaggerated T-cell response"("Epstein-Barr Silver Anniversary,"1989, p.1171). This immune response to EBV infection has been described as "immunologic chaos" (Thorley-Lawson, 1988) and may not be entirely EBV specific. One result of the B cell proliferation is the production of IgM heterophil antibodies. Their immunological function is not known and is considered nonspecific. These heterophil antibodies react with sheep erythrocytes in the classic Paul and Brunnell test and their presence is used to diagnose mononucleosis. Large numbers of cytotoxic/suppressor (CD8) T cells are released into the blood. These are seen microscopically as atypical lymphocytes and may also be used in the diagnosis of mononucleosis. These T cells, although considered as another nonspecific immune response, may actually be an attempt by the immune system to turn off the proliferating B cells.

The immune response to EBV infection is threefold. A cellular response is mounted against virus-infected cells involving both natural killer (NK) and cytotoxic/suppressor T cells. NK cells respond to viral infection singularly and do not require antigenic presentation by B or T lymphocytes. They may destroy virus-infected cells directly or participate in the destruction of antibody-coated infected cells. There is no production of memory NK cells. B cells, as well as macrophages, present antigen to T cells which then produce virus killing interferons and lymphokines. These in turn stimulate more B cell proliferation and differentiation to antibody-producing plasma cells. Both B and T cells produce memory cells which will respond very quickly to any new challenge from EBV. The antibody response to EBV includes the production of IgG and IgM antibodies to the viral capsid antigen (VCA). Both antibodies appear early on during infection. IgM antibodies to EBV are transient but IgG antibodies persist throughout life and are capable of neutralizing virus if infection by EBV recurs. The IgG response to EBV is marked and high titers of IgG-VCA will remain indefinitely. Antibodies to early antigen (EA) appear three to four weeks after the onset of illness and may be transient or persist at low titers indefinitely. The last antibody to appear is produced against the nuclear antigen component (EBNA) of the virus. EBNA antibody may or may not be detected. Possibly, its absence is due to poor humoral response by certain individuals to EBNA (Jones et al., 1985) or incomplete antigenic stimulation of B cells.
during primary infection. Clinical assays for all four antibodies are available and are used in serological diagnosis of EBV infection.

EBV has been identified as the primary cause of infectious mononucleosis. Latent EBV infection is controversially associated with numerous other illnesses. Some of these illnesses are restricted geographically, such as Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC) which occur mainly in central Africa and southern China respectively. Until recently, this virus was considered the cause of chronic fatigue syndrome (CFS) sometimes referred to as "yuppie disease", due to its prevalence in more affluent individuals in the United States. The virus has also been implicated in autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, Sjogren's syndrome and multiple sclerosis. EBV infection has been identified with the generalized lymphoproliferative disease seen in transplant patients and leukoplakia seen in AIDS patients. It has been the suspected cause of Hodgkin's and Graves diseases. The common denominator for all these illnesses is the presence of elevated antibody titers to EBV and/or the recovery of virus or identifiable EBV DNA from patients with these illnesses. Numerous investigators have reported connections between EBV-associated illnesses and defects of cellular immunity. Other investigators have reported connections between EBV-associated illnesses and environmental factors. Below is a brief description of a number of these EBV-associated illnesses which have been connected to cellular immune defects and/or environmental factors.

**Rheumatoid Arthritis**

Patients suffering from rheumatoid arthritis (RA) tend to have higher antibody titers to EBV antigens than do normal individuals. They also show abnormal T cell suppressor activity towards EBV. T cells from these patients are unable to turn off proliferating B cells in vitro, whereas, the addition of T cells from individuals without RA will inhibit EBV-infected B cell proliferation (Depper et al., 1981; Winrow et al., 1988). Some studies have shown reduced action by CD4 cells on CD8 cells (Fawcett et al., 1988). Other studies have indicated that CD8 cells may be normal in number, but are either not stimulated properly or may be dysfunctional (Winrow et al., 1988). It is also suspected that production of interleukin-2 which is cytotoxic to EBV-infected B cells is decreased in patients with RA (Roberts, 1989). The T cell dysfunction observed in patients with RA appears to be specific for this illness and is not found in other autoimmune diseases (Tosato et al., 1981). Otherwise, patients suffering from rheumatoid arthritis show no obvious ill effects from infection with EBV (Thorley-Lawson, 1988; Tosato et al., 1981).

**Nasopharyngeal Carcinoma**

NPC is restricted to certain geographical areas, including southern China, Tunisia and Greenland (Ebbesen et al., 1983; Melbye et al., 1984; Tamada et al., 1984). Individuals with NPC have increased antibody titers to EBV and the tumor cells of these patients also contain EBV DNA (Thorley-Lawson, 1988). It is known too that the soils of these geographical areas contain high levels of nickel, nitrosoamines and diterpene esters which are suspected carcinogens (Ito et al., 1983; Shoa et al., 1988; Wu et al., 1986; Zeng, et al., 1983). These same chemicals induce EBV-infected B cell proliferation which in turn will stimulate T suppressor cell production. Increased numbers of suppressor T cells have been noted in patients with NPC. It is possible that the increased suppressor T cell activity
prevents normal immunologic control of NPC tumor cells (Sundar & Menezes, 1985). However, in an earlier study it was shown that patients suffering from NPC showed reduced suppressor T cell activity (Moss et al., 1983). It is possible that the immune system is responding to increased numbers EBV antibody-producing B cells and NPC tumor cells by expanding the population of cytotoxic/suppressor T cells. However, the T cells being produced may be dysfunctional and can neither control the B cell activity nor destroy the NPC tumor cells.

**Burkitt's Lymphoma**

Like NPC, the association between EBV and Burkitt's lymphoma (BL) is also geographical. Areas of Africa, New Guinea and Sweden have higher rates of BL than other parts of the world (Ohigashi et al., 1985). Over ninety percent of patients with this cancer have recoverable EBV DNA in their tumor cells (Roberts, 1989). However, in a small percentage of these patients no EBV DNA can be recovered and there is no serologic evidence of prior EBV infection. One hundred percent of patients suffering from BL do have the same chromosomal translocation (Thorley-Lawson, 1988). It is this distinction, the presence of the chromosomal translocation in the absence of EBV, which casts doubt on EBV as the causative agent of Burkitt's lymphoma. (Bornkamm et al., 1984; Ito et al., 1983; Whittle et al., 1984).

**Hodgkin's Disease**

Over ninety percent of patients with Hodgkin's disease show elevated titers to EBV antigens. However, as in BL a small percentage of patients with this illness show no exposure to the virus or low antibody titers (Mueller et al., 1989). Decreased T helper/suppressor ratios are reported in many of these patients. As in NPC, it is possible that the increased suppressor T cell activity observed in Hodgkin's disease may act to decrease the body's immune response to tumor cells (Masucci et al., 1984). The elevated EBV titers found in these patients may be due to defective immune response to viral antigens and, therefore, secondary to the illness and not indicative of its cause.

**Multiple Sclerosis**

Elevated IgG antibody titers to EB-VCA viral capsid antigen have been detected in patients suffering from multiple sclerosis (Sumaya et al., 1985). Decreased numbers of suppressor T cells have also been detected in these patients (Craig et al., 1988). Therefore, the increased EBV activity in these patients may be due to the inability of the immune system to check viral expression in B cells (Craig et al., 1983).

**Sjogren's Syndrome**

Sjogren's syndrome is an autoimmune disease affecting the exocrine glands. Defective natural killer cell and suppressor T cell function is involved in its pathology (Fox et al., 1986; Yamaoka et al., 1988). Increased viral shedding from EBV-infected salivary glands of patients suffering from Sjogren's syndrome has been demonstrated (Miyasaka et al., 1989). A resulting elevation of IgG VCA titers has also been observed. Again, increased Epstein-Barr viral activity may be due to faulty immunoregulation of EBV infected cells.
Kawasaki's Disease

Kawasaki's disease is "thought to be a self-limited immunologically mediated vasculitis" (Kikuta et al., 1988). In the paper cited here, increased IgG antibodies to EB-VCA and early antigen are reported. A decreased number of suppressor T cells is also reported, again showing the connection between cellular immune deficiencies and elevated EBV antibody titers.

Chronic Fatigue Syndrome

In the past few years chronic fatigue syndrome (CFS) has become closely identified with EBV in the United States. The illness itself is controversial and, as a syndrome, comprises many symptoms. These symptoms may include fatigue, low-grade fever, headaches, sleep disorders, depression and myalgias that can last for years. The most common symptom of this illness, fatigue, is present in almost all patients. Fatigue is a major symptom in infectious mononucleosis, as well. Furthermore, IgG antibodies to EB-VCA and early antigen are elevated during the course of CFS, implicating EBV as a cause of this illness (Buchwald et al., 1987; Holmes et al., 1987; Merlin, 1986). However, current research is showing that patients who suffer from CFS have cellular immune deficiency problems. The particular deficiency in CFS patients has been shown to involve decreased natural killer cell activity (Caligiuri et al., 1987; Joncas et al., 1989). Since natural killer cells are a first line defense against virally infected cells, decreases of NK cells will lead to increased shedding of viral particles by EBV-infected cells. The resulting humoral response will produce elevations in antibody titers such as antibody to early antigen. Again, the primary cause of chronic fatigue syndrome is unknown. However, it is becoming more apparent that the presence of EBV is secondary to the real cause of this illness. ("Chronicity of Epstein-Barr Virus Infection," 1985, p. 119; Jones et al., 1985; Merlin, 1986; Ritz, 1989; Straus et al., 1985).

It has been clearly established that following primary EBV infection, latent infection remains. This is true whether primary infection occurred as infectious mononucleosis or as a subclinical infection barely noticeable by the infected individual. It is known that viral shedding may occur from the epithelial cells of the oropharynx after recovery from primary infection has taken place and that intermittent viral shedding may continue indefinitely. It is fair to assume that latent infection will be prevented from re-establishing a disease state and becoming a chronic active infection if the immune system of the infected individual is functioning properly. If the virus enters its replicating cycle, both cellular and humoral immune defenses will respond. The former will perform its function by destroying infected cells which express EBV antigen. The latter will lead to increased production of IgG-VCA and EA antibodies. If there is a defect in the cellular immune system, then it would be expected that viral expression would proceed at an increased rate. Similarly, it would be expected that the humoral response would also be significantly increased in order to neutralize and check increased viral replication. Following this logic, it would be conceivable that VCA and EA IgG antibody levels would be expected to increase in illnesses where cellular immune defects exist. In other words, the presence of elevated EBV VCA and EA IgG antibody levels, when primary infection from EBV has been ruled out, may indicate the existence of cellular immune deficiencies. Cellular immune deficiencies involving natural killer and/or CD8 cytotoxic/suppressor cells are of particular interest here.
Illnesses associated with EBV infection are numerous and diverse. They are classified as autoimmune diseases, malignant carcinomas, chronic viral infections, allergies, lymphoproliferative disorders and even headaches. These illnesses are usually chronic, and correlate with elevated levels of IgG-VCA and EA antibodies to EBV. There is increasing evidence for their association with cellular immune defects.

This thesis proposes a model to determine if cellular immune deficiencies are the cause of elevated IgG antibody titers to EBV VCA and EA in patients with EBV- associated illnesses.

Conclusion

It is now well established that most of the human population becomes infected with Epstein-Barr virus. It is also well documented that individuals with various types of the previously mentioned illnesses show increased levels of EBV activity as evidenced by elevated IgG antibody levels to VCA and EA. These illnesses are sometimes described as "EBV-associated". The association could be due to a defect in cellular immunity which permits development of a disease state and at the same time allows reactivation of latent EBV infection.

If one's immune system functions properly, chronic disease should not occur. A properly functioning immune system should also keep a latent infection from becoming reactivated and reintroducing active disease. A virus such as EBV which causes latent infection will coexist with the host and only expresses itself when the immune system of the host is not functioning properly.

If defects in natural killer cell activity and/or cytotoxic/suppressor cell activity occur and chronic illness develops, a corresponding increase in EBV activity will be observed indicating reactivation of latent EBV infection. The increased EBV activity as evidenced by increased antibodies to the virus will most likely be non-pathologic and may be used as a marker to indicate the presence of defective cellular immunity. Observations of serologic data at a large medical reference laboratory using indirect immunofluorescent antibody testing indicate that EBV antibody levels routinely run higher than those of other viruses. The reason for this may be that EBV is more antigenic and enhances a stronger B cell response than other viruses or that EBV, being omnipresent, continuously stimulates antibody production.

EBV has been associated with many illnesses. Realistically, it cannot be the cause of such a diverse number of diseases. Some of these diseases such as Burkitt's lymphoma and nasopharyngeal carcinoma, due to their restricted geographic locales, must be considered to be environmentally induced. It would appear that defects in cellular immunity coupled with cancer causing agents found within the environment are responsible for the high incidence of these cancers in their respective geographic areas. If EBV is the causative agent of these carcinomas then they should be more widespread throughout other parts of the globe. It has been demonstrated that epithelial NPC tumor cells contain EBV DNA. NPC cells are found near the site of primary EBV infection, the oropharynx. Due to the latency of EBV infection it would be expected that EBV DNA would be recovered from epithelial cells of the nasopharynx whether they are cancerous NPC cells or normal tissue. This should hold true for anyone having been infected with EBV. A cancer causing agent causes mutations which
normal immune surveillance cells detect and destroy. If defective cellular immunity is present then neoplasms such as BL or NPC are more likely to develop. In the meantime, independent of the developing neoplasm, but for the same reason the neoplasm develops EBV activity increases. Since the humoral response is independent of the cellular cytotoxic response, the rising level of EBV activity induces elevated levels of antibody to EBV antigens.

This thesis intends to demonstrate that:

(1) EBV is associated with illness and does not cause primary illness except in the instances of infectious mononucleosis and lymphoproliferative disease seen in clinically induced immunosuppression.

(2) The detection of elevated IgG antibody levels to EBV-VCA and, especially, early antigen may be used as markers to indicate the presence of cellular immune deficiencies.

A test model is proposed to test for the presence of cellular immune deficiencies in relation to elevated levels of IgG antibody to EBV. Admittedly, the test model as originally envisioned may have to be expanded to include functional

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