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13. ABSTRACT (Maximum 200 Words)

In this grant, we proposed to generate an EBV infected mammary epithelial system that could be used to characterize the life cycle and oncogenic properties of EBV in this unique tissue. We have been utilizing primary human mammary epithelial cell lines generated in Myles Brown's laboratory. By Fluorescence Activated cell Sorting (FACS), we determined that these cells are positive for the EBV receptor, CR2 and we have been carrying out infection studies to determine the infectability of these cells to EBV. To date, we have found that despite the presence of the CR2 receptor, infectibility is relatively low. This makes transient studies more difficult. We have therefore, begun exploring additional methods for obtaining high infection efficiencies. We believe that we are making encouraging progress which has been facilitated by the funding of this application. We are confident that our studies will lead to the development of important reagents and ultimately further funding in this area of research.

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INTRODUCTION:
EBV is a human herpes virus that infects B-lymphocytes as well as epithelial cells (9, 16, 18). EBV is the causal agent of infectious mononucleosis and is associated with the development of both B-cell and epithelial cell malignancies including the endemic form of Burkitt's lymphoma, post-transplantation lympho-proliferative diseases, AIDS-associated lymphomas, Hodgkin's disease, undifferentiated nasopharyngeal carcinoma (8, 13). EBV has also been linked to some cases of gastric carcinoma (2, 7, 17), T-cell lymphoma (15), and lymphoepithelioma-like carcinoma in the salivary glands, lung, and thymus (4, 14, 19). Although the possible role of EBV in breast cancer has been controversial, more recent publications have provided a potentially interesting correlation between EBV and breast cancer (1, 3, 5, 6, 10-12). This led us to propose investigating this relationship further. We proposed to generate a tissue culture model in which the biology of EBV in mammary tissues could be studies as well as its possible role in the initiation and/or maintenance of breast cancers.

BODY:
Since we found that human immortalized mammary epithelial cells (IMECs) express the CR2 receptor, we believed that we could infect these cells with EBV to address EBV biology and EBV's influence on cell growth/transformation properties. Nevertheless, based on previous work in our lab as well as many other labs studying EBV, it is clear that EBV infection is typically low level despite the presence of CR2 on target cells. We have now addressed the efficiency of EBV infection in IMEC cells. For our initial studies, we generated virus from the B95-8 cell line after several days and high density. Using these conditions, we obtained <1% of cells infected with EBV as measured by immunofluorescene against EBNA1. In our application, we proposed to address the pattern of EBV gene expression during the initial stages of viral infection. Since these studies could be more readily carried out if we were able to obtain higher levels of infection, we carried out further experiments in an effort to obtain a higher infection efficiency. We tested whether concentration of virus generated from B95-8 cells yields higher infection efficiencies but infection efficiencies remained low. We therefore tested an alternative (although more expensive) method in an effort to obtain higher titers of EBV. Treatment of the EBV positive Burkitt's lymphoma cell line, Akata, with anti-Ig yielded significantly higher infection efficiency. Nevertheless, the levels of infectivity were still insufficient to carry out the proposed studies. We are therefore now planning to generate stable IMEC cell lines that express high levels of the CR2 receptor and we believe that this in combination of high titer virus from Akata cells will now allow us to carry out the proposed studies in detail.

We have indicated in the previous report that we obtained encouraging results in soft agar assays and that we might be able to generate cell lines containing stable EBV genomes by growing these colonies up. However, these clones did not expand to sufficient levels indicating that other events besides acquisition of EBV may be required for maintaining growth in soft agar. We plan to carry out these experiments in combination with the introduction of oncogenes that are known to be overexpressed in breast cancer and these studies may shed light on the possible cooperativity between EBV and genetic alterations found in breast cancers.

KEY RESEARCH ACCOMPLISHMENTS:
- Determined that IMEC cells express the EBV receptor, CR2.
- Made progress in developing conditions for higher level infectivity of IMEC cells with EBV.
- Obtained preliminary evidence that EBV enhances growth in soft agar.
- Obtained evidence that long term growth in soft agar requires additional genetic alterations.
- Determined that high level infectivity with EBV requires higher levels of CR2 expression.

REPORTABLE OUTCOMES:
- Any cell line containing EBV plus or minus breast cancer associated oncogenes will be made freely available to the public

CONCLUSIONS:

1) We are making progress on the development of conditions that are suitable for characterizing the EBV gene expression program during initial infection. In addition, we have determined that long term growth of EBV in soft agar requires additional genetic events. Together, these studies have helped us to develop appropriate methods and assays to carry out definitive experiments directed towards addressing the role of EBV in breast cancer.

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