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RECENT ADVANCES ON EPSTEIN BARR VIRUS AND ASSOCIATED DISEASES

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This overview is largely based on papers presented in two international conferences: The 3rd International Symposium on Epstein Barr Virus and Associated Malignant Diseases, Rome, October 1988 and The International Conference on Epstein Barr Virus – the First 25 Years, Oxford, April 1989.

Current research on the Epstein Barr Virus (EBV) and its associated diseases can be broadly divided into:

1) Molecular biology of EBV latency.
2) EBV and epithelial cells.
3) Immunology of EBV infection.
4) Burkitt's lymphoma (BL).
5) Nasopharyngeal carcinoma (NPC).
6) Other EBV-associated diseases.
7) EBV vaccine.

MOLECULAR BIOLOGY OF EBV LATENCY

This is by far the most active area of research on EBV EBV infection immortalises B-lymphocytes resulting in a proliferating lymphoblastoid cell line of transformed B-cells with EBV in 'latency'. During latency, EBV DNA and latent proteins (EBV nuclear proteins, latent membrane protein, terminal protein) are detectable intracellularly. At any one time, only a very small proportion of infected cells progress from latent to virus replicative cycle, resulting in the production of virus structural proteins-viral capsid antigen (VCA), early antigen complex (EA), membrane antigen (MA)-and virus particles.

Research on the molecular biology of EBV latency is focused on:

a) ***Identification of EBV protein expressed during latency.***

The known proteins expressed during EBV latency include six EBV nuclear antigens-EBNA-l, 2, 3a, 3b, 3c and leader protein (LP)-the latent membrane protein (LMP) and terminal protein (TP).

In biopsies of NPC, only EBNA-1 and, sometimes, LMP are expressed. In biopsies of BL, only EBNA-1 is regularly expressed. *In vitro* explants from BL express only EBNA-1 but frequently the other EBNA's and LMP are also switched on upon prolonged *in vitro* culture.

b) ***Biological role of EBV latent proteins.***

Only a very limited number of the functions of EBV latent proteins are known.

EBNA-1 is required for the maintenance of EBV episomes, by binding to the origin of latent replication. EBNA-2 is likely to be important for immortalisation of B lymphocytes by EBV since a deletion in EBNA-2 renders the virus incompetent for immortalisation.

LMP is likely to be an important mediator of EBV-induced lymphoproliferation since expression of this protein in EBV – negative BL cells, as well as in rodent fibroblasts, markedly alters the growth properties, including anchorage dependance and contact inhibition, of these cells.

Experiments to study the roles of latent proteins have been largely done by using deletion mutants of EBV or by transfection experiments. In transfection experiments,' specific DNA sequences are introduced into cells and the resulting effects of expression of the genes observed.

c) ***Identification of the genes coding for EBV-latent proteins.***

The genes are named by their reading frames, i.e. the exact sequences of EBV DNA which code for the specific proteins. For example, BKRF-I is the Bam H, restriction fragment K right reading frame no. 1; it contains the coding sequence for EBNA-I. BYRF-1 is the Bam H, restriction fragment Y right reading frame no. 2; it contains the coding for EBNA-2.

d) ***Control of EBV latency.***

The most important aspect of control of EBV latency is in identifying the gene products that control the shift from latency to the viral replicative cycle.

The BZLF-1 product (dubbed 'Zebra') triggers the replicative cycle of EBV.[2] BZLF-1 can be activated by cellular proteins induced by exogenous agents such as the EBV replicative cycle inducer

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12-0-tetradecanoyl-phorbol- 13-acetate (TPA) or by age. The BZLF-I product induces the expression of BMLF-1 and together these two proteins transactivate other EBV lytic cycle promoters, and initiate a cascade of lytic cycle expression. It is likely that methylation of EBV DNA is an important mechanism in EBV gene control since a strong correlation has been found for the level of inethylation and EBV gene expression.

e) *Modulation of host cellular* ***gene*** *expression.*

EBV immortalisation of B-lymphocytes enhances the expression of B-cell activation markers such as CR2/CD21/EBV receptor and CD23/Blast 2, the B-cell activation antigen.
Cell surface adhesion molecules play an important role in intercellular interactions including recognition by specific cytotoxic T-lymphocytes. Expressions of the intercellular adhesion molecules, LFA-I and LFA-3 (lymphocyte function associated antigens), and intracellular adhesion molecule (ICAM-l) in B-cells have been found to be upregulated by EBV infections. The relevance of such findings to the pathogenesis of EBV-associated diseases is not clear.

EBV AND EPITHELIAL CELLS

EBV DNA has been consistently found in malignant epithelial cells in NPC, but for many years, only B-lymphocytes were shown to have receptors for EBV. It has now been demonstrated by immunocytochemistry using monoclonal antibodies against epitopes of EBV/C3d receptor of B-cells that such receptors are present on cervical and nasopharyngeal epithelia.[3,4] The presence of EBV receptors on epithelial cells was also found to be cell-differentiation-dependent as in B-lymphocytes. It is now believed that EBV infects the less differentiated cells of the basal epithelium, virus replication only occurring as progeny cells approach terminal differentiation. This provides a self-sustaining reservoir of infection in the nasopharynx. The secondary infection of B-lymphocytes which results in the apparent virus carrier status of B-lymphoid tissue is likely to be not required for virus persistence.[5]

IMMUNOLOGY OF EBV INFECTION

The focus is on the identification of target epitopes for EBV-specific T-cells. Most of the promising work has been done using synthetic peptides and EBNA-2 deletion mutants. Interestingly, EBV strain variation has been recently described.[6] Two families of EBV isolates have been characterised based on the antigenicity of the EBNA-2 encoded. EBNA-2 type-A is of 82-87 kilodalton and EBNA type-B 75 kilodalton. Antigenic and sequence variations in the EBNA-3a, -3b and -3c between type-A and type-B strains of EBV have also been reported. The specific differences in EBNA-2 and EBNA-3 are being studied in the hope of identifying targets for EBV-specific cytotoxic T-lymphocytes.[7]

BURKITT'S LYMPHOMA (BL)

BL has a special place in the history of EBV since it was from a BL biopsy supplied by Dr. Denis Burkitt from an African BL patient that EBV was first isolated in Professor M.A. Epstein's laboratory. 96% of all BL have EBV in them. These are the endemic BL (eBL). In sporadic BL (sBL), there is no EBV. However, all BL cells carry a translocation with a consistent breakpoint at chromosome 8, adjacent to the c-myc oncogene. The subsequent translocation results in juxtaposition of c-myc with one of the immunoglobulin complexes resulting in the deregulation of c-myc transcription and constitutive expression of c-rnyc. It has been demonstrated that the deregulated c-myc isolated from BL cells can induce cell proliferation when introduced into normal cells and, in cooperation with other oncogenes such as ras and polyoma large T, can result in full blown malignant transformation.

It is thought that EBV infection of B-cells carrying the c-myc translocation leads to continued growth and subsequent proliferation. Interestingly, there is a strong correlation between eBL and the areas of hyper- and holo-endemic malaria. It has been suggested that malaria is a potent activator of B-cells, providing an increase in the pool of B-cells available for the appropriate translocation.

Much of the current research on BL is focused on the effects of c-myc on the growth characteristics of BL cells and the control of gene expression in BL biopsies and BL–derived cell lines. Methylation of EBV genes has been found to be one of the control mechanisms for BL gene expression.

NASOPHARYNGEAL CARCINOMA (NPC)

The large majority, if not all, of NPC biopsies contain EBV DNA positive malignant epithelial cells. The expression of EBV genes in NPC biopsies has been studied by immuno-

blotting of polyacrylamide-separated proteins of biopsies.[8,9] Among the EBV-latent genes expressed in transformed B-lymphocytes, only EBNA-1 is regularly expressed. LMP is occasionally expressed. Much work is also done using a nude-mice passaged EBV-positive NPC cell-line, C15.[10] In C15, the pattern of expression of EBV-latent genes is similar to that seen in NPC biopsies. The malignant epithelial cells in C15 express increased amounts of cellular proteins including HLA class II DR, DP and DQ molecules, and also antigens associated with normal B-cell activation such as CD23 and CD40. No clear picture on activation of oncogene in NPC has emerged.

Seroepidemiology studies conducted in China has demonstrated that IgA against EBV VCA and EA can be used for early detection of NPC since normal individuals with elevated titres were found to be at a significantly higher risk of developing NPC.[11] A report from Hong Kong,[12] based on a case-control study on two hundred and fifty cases of NPC below age 35 years, mdicated that 95% of these young NPC's can be attributed to consumption of Cantonese-style salted fish during childhood.

Elevated titres of IgA against EBV VCA and EA have traditionally been titrated by the technique of indirect immunofluorescene with EBV-positive cells as target antigens. Recently, titration of antibodies by enzyme-linked-immunosorbent-assay (ELISA) against proteins expressed from cloned fragments of EBV DNA or against synthetic peptides of EBV antigens has been employed. This immunoassay promises to be more rapid, economical, sensitive and specific than current methods using immunofluorescene and immunoperoxidase.

OTHER EBV-ASSOCIATED DISEASES

EBV is believed to have an etiological role in both eBL and NPC. In immunocompetent persons, EBV infections lead to self-limiting infectious mononucleosis (IM). EBV is an ubiquitous virus and, once exposed, a person carries EBV DNA positive cells latently for life with the possibility of reactivation during immunodeficiency. In patients with the rare X-linked lymphoproliferative syndrome (XLP), EBV infection is often fatal. EBV reactivation can occur in immunosuppressed post-transplant patients, giving rise to a form of mononucleosis syndrome. This may progress to widespread infiltration of vital organs or to the development of extranodal solitary lymphomas. In patients with the acquired immunodeficiency syndrome (AIDS). undifferentiated lymphomas with EBV-genome positive cells have been reported with increasing frequency. Oral 'hairy' leukoplakia (OHL) is a recently described lesion of the tongue occurring in association with AIDS. In OHL, active EBV replication occurs in the epithelial cells. It is the only *in vivo* situation where intact EBV particles together with EBV lytic cycle antigens (VCA, EA, MA) have been found.[13] Elevated antibodies to EBV antigens have been reported in Chronic Fatigue Syndrome, however, titres of antibodies are also elevated against other viruses. Elevated antibodies to EBV structural antigens have also been demonstrated in a significant number of patients with rheumatoid arthritis and systematic lupus erythernatosus.[14]

DEVELOPMENT OF VACCINE

Vaccination against EBV as a prevention of IM, BL and NPC has been suggested for many years by Professor M.A. Epstein. It has now been shown that the EBV membrane protein GP340 together with the adjuvent N-acetyl-muramyl-L-threonye-D-isoglutamine is able to protect cottontop tamarins against EBV-induced lymphoma. The cottontop tamarin is the only animal model for EBV-induced lymphoma. Recently, the isolation of large quantiries of purified GP340 has been greatly improved by the use of anion exchange and gel filtration using a fast-protein-liquid-chromatography system. A small-scale trial has now been initiated in Oxford. United Kingdom, to determine if the vaccine is effective in preventing IM. The next step involves vaccinating males in XLP families. If the trials are successful. the vaccine will be used in Gambia to determine the effects on the incidence of BL over the next decade. The final goal of the EBV vaccination programme is to vaccinate EBV-negative babies with the aim of reducing the incidence of NPC. The high incidence of NPC makes such a proposition worthy of consideration.

In order to raise large quantities of pure EBV GP 340, research has been on going to express GP340 in various systems including bacteria. vaccinia and insect viruses. besides the modifications to purification procedures used to extract GP340 from membrane of EBV infected cells.

EBV was discovered 25 years ago. We are only now beginning to understand some of the immunobiology of this extremely complex virus

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