**Induction of Epstein-Barr Virus Antigens in Human Lymphoblastoid P3HR-1 Cells with Culture Fluid of *Fusobacterium nucleatum*¹**

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**ABSTRACT**

Epstein-Barr virus-associated early antigen and viral capsid antigen were efficiently induced in human lymphoblastoid P3HR-1 cells with culture fluid of *Fusobacterium nucleatum*, a member of the indigenous microbial flora of the human host. This finding may suggest a new approach to assess the possible role of the “cofactor(s)” in the etiology of Epstein-Barr virus-related diseases.

**INTRODUCTION**

It is now widely accepted that EBV² is a viral agent ubiquitously distributed among humans, regardless of ethnic and/or socioeconomic situations. EBV infection takes place either very early in life or presumably at adolescence at the latest, and the viral genome remains in human lymphatic cells in a repressed or “dormant” state throughout life (1). For such an agent to bear any relevant role of etiological significance, as it has been long suspected to do in BL and NPC, we assume that the EBV induction phenomenon (3) could well be the first step in a chain of events which ultimately lead to the emergence of cancer.

Recent data from the laboratory show that EBV can be induced in EBV genome-carrying human lymphoblastoid cells by treatment with chemicals such as halogenated pyrimidines (2, 8), n-butyrate (6), tumor-promoting phorbol diesters (10), and also by antibodies to human IgM (9). We have recently found that not only n-butyric acid but also other short-chain fatty acids such as n- and i-valeric acids possess the capacity to induce EBV. These short-chain volatile fatty acids are known as the fermentation products of the indigenous microbial flora of humans, which include genera of *Fusobacterium*, *Bacteroides*, *Clostridium*, and other anaerobes.

Thus, in our attempt to search for unknown effective EBV inducers in the environment, we first tested the culture fluid of *Fusobacterium nucleatum*, which is commonly isolated from the oral cavity and upper respiratory tract and which accumulates an abundance of n-butyric acid in culture medium (7).

**MATERIALS AND METHODS**

A strain of *F. nucleatum* B-1 from our departmental culture collection was inoculated in 25 ml broth medium [trypticase, 1.5 g/dl; heart extract, 0.5 g/dl; yeast extract, 0.3 g/dl; NaCl, 0.2 g/dl; Na₂HPO₄, 0.2 g/dl; KH₂PO₄, 0.1 g/dl; glucose, 0.5 g/dl; sodium pyruvate, 0.5 g/dl; L-cysteine HCl, 0.03 g/dl; hemin-vitamin K₃ solution, 1.0 ml; Tween 80, 0.025 g/dl; (pH 7.2)] and cultivated at 37°C for 5 days by the steel wool method anaerobically (CO₂:N₂, 10%-90%). The culture fluid was then passed through a Seitz bacterial filter and was used as samples for EBV induction trials. Uninoculated broth medium treated in the same way served as the controls. The amount of fatty acids in the culture fluid was assayed by conventional procedure (5), using an Hitachi Model 163 gas chromatograph. The assay curve of *F. nucleatum* culture fluid used in the present experiment is shown in Chart 1. The fluid contains approximately 26 mM butyric acid and 24 mM acetic acid.

For EBV induction trials, the bacterial culture fluid and the uninoculated control medium were diluted 2-fold with Roswell Park Memorial Institute Tissue Culture Medium 1640 from 5 to 80 times, and P3HR-1 cells (3) were suspended in (a) 2-ml volume of each dilution at 1 x 10⁶ cells/ml and cultured for 96 hr at 37°C. The immunofluorescent staining of the cells in each

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³ The abbreviations used are: EBV, Epstein-Barr virus; BL, Burkitt’s lymphoma; NPC, nasopharyngeal carcinoma.

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Chart 2. Induction of the EBV-associated antigens, early antigen (EA) and viral capsid antigen (VCA), in human lymphoblastoid P3HR-1 cells with F. nucleatum culture fluid. a, assays for EBV early antigen; b, assays for EBV viral capsid antigen. The curves shown are induction curves of P3HR-1 cell samples treated with F. nucleatum culture fluid 10 (●), 20 (●), 40 (▲), 80 (○) times. D, P3HR-1 cell samples treated with uninoculated broth medium 5 times. The curves for samples treated with culture fluid 5 times (data deleted) were closely superimposed on the curve of samples treated with culture fluid for early antigen 20 times and on that of samples treated with culture fluid for viral capsid antigen 40 times. The results for samples diluted with uninoculated control broth 10 to 80 times did not differ significantly from the curve of the samples diluted 5 times as described above.

dilution was carried out on cell samples taken at 48 hr and 96 hr.

RESULTS

The most efficient induction was achieved with the 5-day culture fluid of F. nucleatum (n-butyric acid content, 2.6 mm) diluted 10 times. The maximum rate of response obtained was 59.6% (48 hr) for the early antigen and 66.2% (96 hr) for the viral capsid antigen. These figures were in close approximation with the ratio for the optimal concentration of n-butyric acid of a purely chemical source. As illustrated in Chart 2, the decline of the induction curves at higher dilutions (20, 40, and 80 times) was nearly proportional to the dilution factor and therefore, dependent on the n-butyric acid content. The uninoculated broth used as the control showed no sign of induction. As can be seen from the chromatographic data (Chart 1), the control broth did not contain any n-butyric acid but did contain approximately 10 mM acetic acid, which probably was derived from pepton or other ingredients during the autoclaving. This amount of acetic acid had no effect on EBV induction.4

CONCLUSION

F. nucleatum is a member of the indigenous microbes of humans, and the sites where it is commonly isolated are closely related to the regions where BL and NPC occur. The fact that it is an obligatory anaerobe also is of importance. In this context, further investigations to establish the efficacy of these microbial products as EBV inducers and to further assess their possible role as an etiological "cofactor(s)" for diseases such as BL and NPC are currently being conducted in our laboratory.

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