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Presence of Epstein-Barr Virus in Cutaneous Lesions of Mycosis Fungoides and Sézary Syndrome

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It has been suggested that prolonged antigenic stimulation contributes to the development of epidermotropic cutaneous T cell lymphoma (CTCL), mycosis fungoides and Sézary syndrome, characterized by a cutaneous infiltration of proliferating helper T cells. Since Epstein-Barr virus (EBV) antibodies were increased in CTCL sera, we investigated a possible etiologic role for EBV in epidermotropic CTCL by looking for the EBV genome in 25 cutaneous biopsies of mycosis fungoides or Sézary syndrome and 12 reactional inflammatory skin lesions. The use of a non-isotopic in situ hybridization procedure based on the detection of Epstein-Barr encoded RNAs with biotinylated oligonucleotide probes (EBER) revealed 32% of the lesions with CTCL to be positive for EBV (3 in dermis, 3 in epidermis, 2 both in dermis and epidermis), as compared to no detection of the EBV genome in the reactional inflammatory skin lesions. Moreover, a combined hybridization (EBER probe) and immunochemistry technique (anti-CD3 or anti-Kil monoclonal antibody) permitted the identification of EBV in T cells of dermis and in keratinocytes, respectively. The identification of EBV in epidermotropic CTCL suggests that this virus could play a role in the development of these CTCLs, either as an etiological agent or more probably as a chronic activating agent. Indeed, the infection of keratinocytes by EBV could activate them and so induce the production of in situ cytokines (IL1a, IL6, TNFa) playing a role in the development of tumoral infiltrate. ***Key words: cutaneous T cell lymphomas; EBV.***

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Most cases of mycosis fungoides and Sézary syndrome are of the CD4 phenotype, and most cells belong to the memory CDw29 subpopulation (1), arguing for the role of a chronic antigenic stimulation in the development of these epidermotropic cutaneous T cell lymphomas (CTCLs). In this context, oncogenic virus including Epstein-Barr virus (EBV), could play a role in the development of this disease.

Increasing evidence indicates that EBV has an important etiological role in the development of Hodgkin's disease (2–7).

In addition, EBV DNA has been identified in T cell lymphoma (8-13) with activated phenotype (14–15) and CD30 large anaplastic lymphomas (16). And recently, it has been reported that the level of antibodies against EBV nuclear antigen is increased in the sera of patients with epidermotropic CTCL compared to a control population (17).

In this context, we conducted a study to screen for EBV in cutaneous lesions from mycosis fungoides and Sézary syndrome. In order to conduct this study, we used a recent sensitive in situ hybridization technique (18) based on the detection of Epstein-Barr encoded RNAs (EBER), using a biotinylated oligonucleotide probe, which has already been used for the detection of EBV in Hodgkin's disease (19). Moreover, this technique allowed an identification of type of infected cells.

MATERIALS AND METHODS

Patients

Twenty-five patients were studied: 18 with mycosis fungoides at different stages defined by the Scandinavian mycosis fungoides study group (20) (stage II: 15, stage IV: 3) and 7 with Sézary syndrome. The patients were 15 men and 10 women, the average age was 58 (range 22–72), and for each patient the diagnosis was based on clinical, histological and immunohistochemical criteria. No CD30-positive T lymphomas or primary non-epidermotropic CD30-negative T lymphomas were included.

No local therapy was used for at least 2 weeks, nor any therapy for at least 4 weeks.

Two cutaneous biopsies (diseased and normal skin with histological control) and one venous puncture were performed for each patient. For mycosis fungoides all the biopsied lesions were either infiltrated plaques or tumoral lesions.

Controls

Control skins were obtained from 12 subjects with inflammatory skin lesions: 6 patients suffered from atopic dermatitis and 6 from psoriasis. There were 10 men and 7 women with an average age of 55 (range 42–70). For each control patient, two cutaneous biopsies were also performed with a histological control (one in normal skin and one in diseased skin).

Immunological study

The phenotype of the epidermotropic CTCL and reactional inflammatory skins was determined by an indirect immunofluorescence technique on frozen sections using the following monoclonal antibodies (MoAb) goat anti-mouse IgG2: CD2, CD3, CD4, CD8 (Immunotech*).

In situ hybridization

An EBV RNA in situ hybridization was performed using a 30-base oligonucleotide complementary to a portion of the EBER gene. This region of the EBV genome is transcribed in latently infected cells. FITC labelled probes were used; the procedure for the in situ hybridization technique has already been described (21). Briefly, the slides were deparaffinized, dehydrated, predigested with proteinase K (50 μg/ml), prehybridized and hybridized over night at a concentration of 25 ng/μl by probe. After washing, detection was accomplished using purified rabbit F(ab') anti-FITC antibody labelled with alkaline phosphatase system to detect the hybridization signal (in situ hybridization detection system K046, DAKO*). A dark blue color in the nucleus was considered as positive.

Although a sense strand oligonucleotide could not be used as a negative control, substitution of the probe with two other oligonucleotides showed no similar staining. A known EBV-positive undifferentiated nasopharyngeal carcinoma served as a positive control and EBV-negative lymphoid tissue served as a negative control.

The results are expressed in the percentage of EBV DNA-positive cells in dermis (negative: –, less than 25%: +, 25 to 50%: ++, 50 to 75%: +++, more than 75%: ++++).

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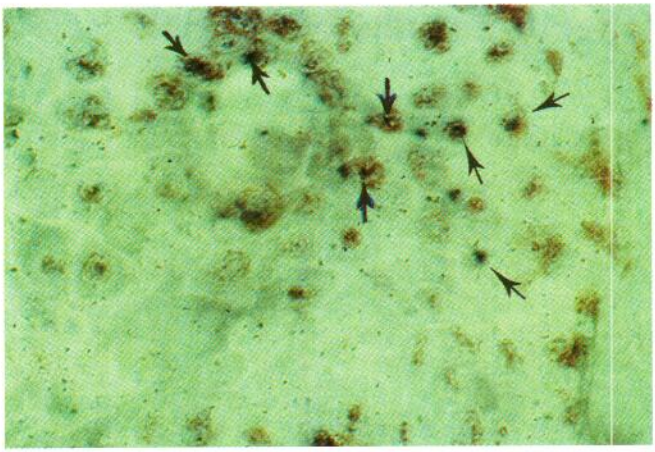

Fig. 1. The combined immunochemistry (CD3 monoclonal antibody) hybridization technique (biotinylated EBV probe) on frozen sections revealed CD3-positive cells (brown color) with EBV RNA (nuclear dark blue colour) in dermis (magnification ×40).

Combined in situ hybridization and immunochemistry

In cases showing positive hybridization for EBV, the phenotype of infected cells was determined by using double labelling (immunochemistry and in situ hybridization) either on frozen sections (anti-CD3 – EBER probe) or deparaffinized sections (KL1 monoclonal antibody – EBER probe). Anti-CD3 monoclonal antibody (Dako) was used to characterize T cells and KL1 monoclonal antibody (Immunotech*) the keratinocytes.

The in situ hybridization was performed according to the same procedure as outlined earlier and was followed by the immunohistochemical technique. Slides were incubated with normal swin (1/10) for 10 min. After washing, monoclonal antibody, either anti-CD3 diluted 1/3 or KL1 diluted 1/5, was added and labelled with goat anti-mouse IgG conjugated with peroxidase (1/10) for 30 min at room temperature. Finally, after washing chromogene AEC (amino ethyl carbazole) Dako* was used, inducing a brown coloration.

RESULTS

Immunological study

The immunological study confirmed the predominant CD4+, CD2+, CD3+ phenotype of the dermal infiltrate both in mycosis fungoides, Sézary syndrome and inflammatory skin lesions. This subpopulation continually represented more than 75% of the dermal infiltrate.

In situ hybridization

Out of the 25 cases of CTCL examined, a nuclear hybridization signal was seen in 8 (32%) cases, corresponding to 5 stage II, 1 stage III, 2 stage IV. In 3 patients an EBV hybridization signal was present only in dermis, 3 only in epidermis, and 2 both in dermis and epidermis. In dermis, the percentage of infected cells was variable (3 +, 1 ++, 1 +++). Only one case of Sézary syndrome (stage III) had detectable EBV RNA and only in dermis, with a low percentage of infected cells (+). The double-labelling study (in situ hybridization-immunochemistry) showed expression of CD3 on the membrane of EBV-positive cells in dermis (Fig. 1), and in a similar manner in epidermis EBV RNA was associated with KL1-positive cells, more often in spinous cell layers (Fig. 2).

In order for us to confirm the specificity of the EBV-positive signal, the sections were subjected to RNAse treatment before hybridization. This treatment completely abolished the signal.

EBV RNA detection was always negative in normal skin (mycosis fungoides, Sézary syndrome and control subjects) and in diseased skin of control subjects.

DISCUSSION

In the present study, we have shown the presence of the EBV genome in 8 (32%) cutaneous lesions of 25 epidermotropic CTCL, using a non-isotopic technique. In situ hybridization is considered as less sensitive than PCR, but it provides morphologic identification of the EBV-infected cells (23), which was one of the aims of our study. However, in situ hybridization detecting EBV EBER region, which is used in our study, has been demonstrated to be a highly sensitive method for the detection of EBV RNA in tissue sections (4), and finally, unlike biotin, FITC labelling gives minimal background for immunochemistry as it does not occur in mammalian tissues (23). In this way, we have identified EBV RNA both in the epidermis and dermis of cutaneous lesions of CTCL. Nevertheless, the double labelling studies successfully demonstrate, firstly, in epidermis the infection of keratinocytes by EBV with detection of EBV RNA in KL1 positive cells and, secondly, in dermis the infection of CD3-positive T cells. EBV detection appears specific to CTCL skin lesions because of the lack of EBV expression in inflammatory skin lesions and in normal skin of CTCL.

EBV is a lymphotropic herpes virus, well recognized for its oncogenic properties. However, in lesions of CTCL it could only play the role of a silent passenger or a modulating factor. Indeed, EBV identification in cutaneous lesions could only reflect an immune deficiency of these patients and an increased viral burden induced by another factor (virus: HTLVI?) in a manner similar to the emergence of Burkitt lymphoma. In this context, it is interesting to point out the fact that EBV was detected in epidermis. Indeed, recently, a modulating effect of

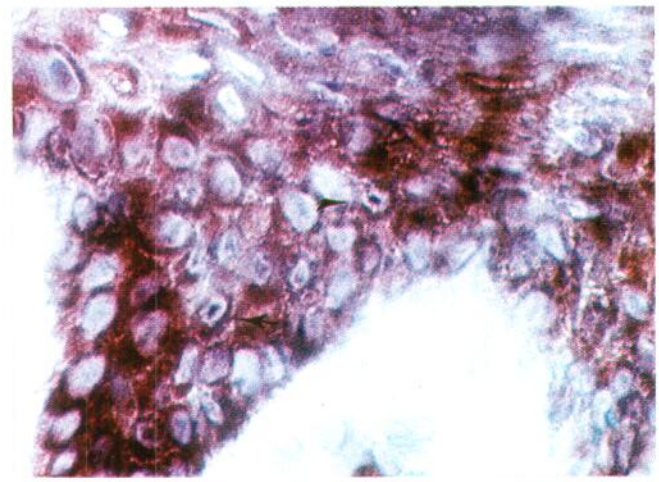

Fig. 2. EBV RNA was detected in keratinocytes in 5/25 cutaneous lesions (magnification ×40) using a combined immunochemistry (KL1 monoclonal antibody) and hybridization technique (biotynulated EBV RNA probe) on deparaffinized sections.

EBV on the production of IL5 mRNA in monocytes has been demonstated (24). In a similar manner this virus could induce an activation of keratinocytes with production of epidermal cytokines as IL-1a, IL-6, and TNFa, which could play an important role in the activation of the CD4 dermal infiltrate in CTCL. The infection of keratinocytes by EBV could be made easier by the expression of CD21 (CR2) antigen, which is the EBV receptor on B lymphocytes, having recently been detected on these keratinocytes (25).

Therefore, the mechanism of EBV entry into T cells is still unknown. Indeed, T lymphocytes, as well as thymocytes in which EBV infection has already been shown (26), do not ordinarily express CD21 (CR2) antigen, or merely express it at an early stage of their differentiation.

In conclusion, the demonstration of the EBV genome in mycosis fungoides and Sézary syndrome by in situ hybridization, both in keratinocytes and T cells of dermis, raises the hypothesis of an activating effect of this virus in the chronic antigenic stimulation of the dermal infiltrate. Further studies are now needed to investigate the significant role of this virus in the development of CTCL.

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