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EPSTEIN-BARR VIRUS ANTIBODY TITRES IN MYCOSIS FUNGOIDES

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Abstract. Sera from 9 patients suffering from mycosis fungoides (infiltrative or tumour stage) and from 16 age-matched normal controls were tested for Epstein-Barr virus antibody titres. Antibodies were found in sera of all of the MF-patients and sera of 15 of the controls. The geometric mean titre in MF-patients was 1 : 640 and 1 : 398 in the controls. The difference is not significant. It is concluded that EB-virus infection may not have any etiological significance in mycosis fungoides.

Mycosis fungoides (MF) is a malignant polymorphic lymphoma predominantly affecting the skin. The course of the disease is variable but three different stages can usually be distinguished: The first stage consists of a non-specific inflammatory rash which resembles chronic eczema, parapsoriasis or poikiloderma. The presenting symptom is pruritus, and the stage lasts for several years. The second stage is characterized by the appearance of inflammatory plaques characterized by a multiplicity of cell types among which are malignant cells of reticulo-endothelial origin. In the third stage numerous tumours appear here and there on the skin. More seldom seen are the erythrodermic and d'emblée forms of the disease. By the time of death one or several internal organs are often involved (1).

The etiology and pathogenesis of MF are completely unknown. Viral etiology has been suggested since transfer of the disease from man to guinea pigs and mice has been claimed by inoculation of tumour tissue (12, 14) and since high viral antibody titres have been found in other kinds of lymphomas. Antibodies to the Epstein-Barr (EB) virus have been found to be significantly higher in sera from patients with Burkitt's lymphoma and nasopharyngeal carcinoma. Additional evidence of the possible etiological role of EB-virus in both these diseases has been demonstrated by detecting EB-viral genome in tumour biopsies taken from patients with Burkitt's lymphoma and nasopharyngeal carcinoma (10, 13). EB-virus have also been shown to be causally related to infectious mononucleosis (4, 8). Raised EB-antibody titres have lately been reported also in sera of patients with sarcoidosis (5, 9) and in Hodgkin's disease (7). This study was initiated in order to find out whether EB-virus antibody titres and thus EB-virus infections might shed any light on the etiology of mycosis fungoides.

MATERIAL AND METHODS

Human serum samples. Serum samples from 9 patients with MF and 16 control patients of the same age were obtained in autumn 1971 in the Department of Dermatology, University of Turku. No patients who were suspected of having any malignancies, infectious mononucleosis, infections caused by herpes simplex virus or cytomegalovirus were accepted as controls.

Immunofluorescence test

Cells. The Silfere Burkitt lymphoma cell line (kindly supplied by Dr Arne Svedmyr, The Virus Department of the Central Bacteriological Laboratory of Stockholm, Sweden) was used for the test. Cell lines were propagated in Eagle basal medium supplemented with 10% fetal calf serum. Prior to use in the immunofluorescence test, about 10^6 cells per millilitre were placed in Eagle basal medium without arginine, supplemented with 10% fetal calf serum. The cells were incubated at 35°C with 5% carbon dioxide for 10 days. After this procedure the Silfere line contains 1–3% EB-virus positive cells. The cells were harvested by low speed centrifugation and washed twice with phosphate-buffered saline solution (BPS), pH 7.4. The cell pellet was mixed with a small amount of PBS and gently suspended with a Pasteur pipette. Three small drops of this suspension were placed on a microscope slide and dried. The microscope slides were fixed in fresh acetone at room temperature for 10 minutes, air-dried and stored at −20°C until used.

Table I. *Epstein–Barr titres in patients suffering from mycosis fungoides and in a series of age-matched control patients*

Sex and (symbol)	Age (y.)	Duration of the disease (y.)	Stage of MF: diagnosis of the control patients	EB-titre
Mycosis fungoides:				
♂ (Bo)	55	3	II, infiltrative plaques	1:640
♂ (Ki)	61	5	II, infiltrative plaques	1:320
♂ (Ti)	64	2	II, infiltrative plaques	1:320
♂ (Jä)	89	2	III, tumours	1:640
♀ (Su)	57	6	II, infiltrative plaques	1:640
♀ (Ra)	70	7	III, tumours	1:320
♀ (Tu)	71	5 (mo.)	III, tumours	1:320
♀ (Ne)	85	4	III, tumours	1:2560
♀ (Ra–Al)	85	3	III, tumours	1:2560
Controls:				
♂ (Ho)	50	4	Eczema corporis	1:000
♂ (Le)	55	1	Eczema man. amb.	1:640
♂ (Jo)	61	4	Eczema cruris	1:320
♂ (Vu)	69	13	Psoriasis vulgaris	1:1280
♂ (Ma)	71	4	Eczema infectiosum	1:320
♂ (Ka)	72	33	Ulcera varic. crur.	1:640
♂ (La)	72	2	Erythrodermia psoriatica	1:320
♂ (Ms)	73	4	Erythrodermia	1:320
♂ (Mj)	87	1	Pemphigoid	1:160
♀ (Ru)	66	9	Dermatitis herpetiformis	1:160
♀ (Ne)	71	1	Eczema allerg. man.	1:1280
♀ (Le)	72	10	Ulcus crur. post-thromb.	1:160
♀ (Nu)	74	1 week	Herpes zoster	1:640
♀ (Yl)	75	3 (mo.)	Eczema allerg.	1:1280
♀ (Er)	78	1	Eczema man. amb.	1:320
♀ (Sa)	81	4	Ulcus cruris	1:320

Test procedure. The indirect immunofluorescence test was performed as developed by Henle and Henle (3) with the following minor modifications. The microscope slides containing the fixed Silfere cells were overlaid by heat-inactivated serum at a 1 : 10 or higher dilution. Slides were incubated at 37° for 45 minutes and washed for 10 minutes in PBS with three buffer changes. After staining with antihuman IgG conjugate (National Bacteriological Laboratory of Sweden) in dilution of 1 : 80 for 45 minutes at 37°C the slides were washed as before, mounted with carbonate-buffered glycerol, pH 8.5 (6) and overlaid with cover glasses. The dilution of 1 : 80 of the conjugate provided about 8 units of fluorescent antibodies, since the conjugates could be diluted 1 : 640 and still yield detectable immunofluorescence. The slides were examined with Ultraphot II microscope using an interference primary filter and a fitting secondary filter (11) with Osram 12 V 100 W Halogen lamp which is very convenient to use with an interference primary filter in the titration of sera and gives as high antibody titres as the conventional HBO-200-W lamp. All serum samples were titrated by reacting serial 4-fold serum dilution with Silfere cells. The brilliance of fluorescence was graded from − to ++, and the last serum dilution yielding a ± reading was taken as the endpoint. If the last positive serum dilution gave a stronger than ± reading, the endpoint was interpolated as halfway between this and the negative fourfold dilution. Although the grading of fluorescence is obviously subjective, the results of titration were closely reproducible when the same sera were titrated at different times.

During titrations, the coded slides were randomized and read blind. Each test included a titration of a standard antibody-containing human serum. Negative controls for fluorescence staining included the use of unreactive human serum and the staining of Silfere cells with the antihuman IgG at the dilution used in titration.

Stability of the test. A known serum of medium titre was tested for reactivity against Silfere cells on 37 different days. A titre of 1 : 160 was obtained 13 times, 1 : 320 on 3 occasions and 1 : 80 21 times.

Sensitivity of the test. In order to compare sensitivity of this immunofluorescence test for EB-antibody with that previously described (2) Arne Svedmyr, M.D. provided us with a positive serum that gave us the same titre as in his laboratory.

RESULTS

The data on the sex, age and clinical stage of the patients as well as of the control patients and on the EB-antibody determinations are given in Table I. The sera from all 9 patients with MF contained antibodies against EB-virus. One of the sera of the 16 control patients did not contain EB-antibodies. The geometric mean titre was 1/640 in the sera from patients with MF and 1/398 in the control group. The difference is not statistically significant. The highest titres, two sera with a titre 1 : 2 560, were, however, found in the MF patients in the tumour stage.

DISCUSSION

This result reveals that the severity or stage of mycosis fungoides does not correlate with the Epstein-Barr virus antibody titres. This result could mean either that EB-virus is not involved in the etiology of mycosis fungoides, or that patients suffering from MF do not respond to EB-virus infection with normal antibody formation. The latter alternative would mean that the MF patients are immunologically defective. Clinical

and experimental studies on antigenic sensitization have revealed, however, that there is no impairment of the circulating antibody response until late in the course of the disease. Similarly, studies with contact sensitizers and skin graft studies have revealed that there is no marked impairment of the delayed hypersensitivity of MF patients either (1). Therefore, the normal antibody titres suggest that patients suffering from MF are not more liable to EB-virus infection than are healthy control persons of the same age group and that EB-virus infection does not seem to have any etiological significance in mycosis fungoides, as has been suggested to be the case in some other forms of lymphomas.

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