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ORIGINAL CONTRIBUTIONS

AETIOLOGY OF RECURRENCE OF PENILE WARTS BY TUBE LEUCOCYTE ADHERENCE INHIBITION TECHNIQUE

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The presence of cellular immunity against penile warts was examined by the tube leucocyte adherence inhibition (T-LAI) assay. Peripheral blood leucocytes (PBL) from six patients with penile warts gave a positive reaction with the antigens prepared from the whole extract of penile warts but not with the antigen prepared from normal penile skin. Adherence of PBL from normal donors did not differ significantly irrespective of whether the antigens were from warts or normal penile skin. All 6 patients responded well to an antigenic extract of warts. LAI reactivity diminished at the first month after removal of growth and gradually reappeared by the second month of removal and never declined till the third month follow up. This indicates some possible reason of recurrence of penile warts.

Key words : Warts, Recurrence, T-LAI technique.

To assess the cell mediated immunity, the tube leucocyte adherence inhibition (T-LAI) technique, introduced by Halliday and Miller[1] is being successfully used in studies of immunity against various types of tumours. This assay is based on the findings that non-sensitised leucocytes from tumour patients or control subjects adhere to glass, whereas leucocytes from tumour patients only but not from the control subjects when mixed in vitro, with antigenic extracts of tumours of the same histological type, undergo a diminution in their normal adherence to glass surface.[2]

In this study, the T-LAI assay has been used to assess the immunity in cases having penile warts. A panel of six wart antigens and six control antigens from the penile skin were prepared, to test adherence of PBL from wart patients and from healthy subjects.

The adherence of PBL of patients was also examined at different periods after excision of their warts. In the present study, the whole extract of warts has been used. Hence, the extract consists of both the viral and tumour antigens.

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Materials and Methods

The penile wart tissues were collected from six patients by excision. Fibrous tissue was dissected away from the material and the specimen was finely minced with sharp scissors. From each patient approximately 4-5 gm of tissue was collected and placed in 5 volumes of ice-cold PBS and homogenized for one minute over a period of 12-15 minutes at 10,000 rpm in a homogenizer under cold condition. The homogenate was centrifuged at 10,000 g for 30-40 minutes and the supernatant was separated and preserved in 0.5 ml aliquots at—20° C.

Following the same procedure, 6 control antigens were also prepared and preserved in the same way.

The protein concentration of both the control extract and the wart extract was measured and expressed as mg/ml for future reference. From each aliquot, 0.1 ml of the extract was added to certain designated tubes containing the standard quantity of 4×10^6 PBL in 0.5 ml of Eagle's minimum essential medium (MEM).

A 5 ml sample of heparinized (100 units/5 ml) venous blood was collected from the cubital vein of each patient and the control, and again from the patients after excision of their warts at monthly intervals upto the third month. The samples were placed in test tubes and incubated vertically at 37° C in humidified atmosphere of 5% CO_2 for 2 hours.

The resulting leucocyte-rich plasma fraction was aspirated and centrifuged at 200 g for 5 minutes, the cell-free plasma was then removed and discarded. The cell button was then suspended in an ice-cold isotonic Tris buffered NH_4Cl (3-4 ml) solution by repeated pipetting and left at 4°C for 15 minutes in order to lyse contaminating erythrocytes. The leucocytes were then washed with MEM and centrifuged. The supernatant was removed and discarded. The cells were then washed twice in the same medium and resuspended at a concentration of 4×10^7 cells/5 ml of medium.

The LAI assay was a tube modification[9] of the method described by Halliday and Miller .[1] Antigen induced T-LAI was tested in 20 ml test tubes. The tubes were arranged in 3 sets :

(A) Cells (0.5 ml i. e. 4×10^6 cells) + 0.1 ml medium (MEM)

(B) Cells (0.5 ml i. e. 4×10^6 cells) + 0.1 ml wart extract

(C) Cells (0.5 ml i. e. 4×10^6 cells) + 0.1 ml penile skin extract.

Each tube was brought to a final volume of 1.0 ml by adding 0.4 ml of MEM. The suspension in each tube was thoroughly mixed and the tubes were then incubated at 37°C overnight in a horizontal position so that the contents covered 3/4 of the lower surface of each tube. On the following day, the number of non-adherent cells/ml was counted in a haemocytometer after thoroughly mixing the cells. The results were expressed as : (1) Percentage of non-adherent cells, and (2) Non-adherence index (NAI).[4] (NAI is defined as the percentage of non-adherent cells in the presence of wart antigen minus the percentage of non-adherent cells in the presence of penile skin antigen divided by the percentage of non-adherent cells in the presence of penile skin antigen).

Results

The results have been depicted in table I.

Table I. Results of Tube-LAI activity in normal PBL donors and in patients with penile warts, before removal and at different periods after removal.

PBL donors	LAI (% of non-adherent cells) with		
	Penile skin tissue (Mean±S D)	Wart tissue (Mean± S D)	Statistical value
Normal PBL donors	34.60±2.68	34.78±1.93	t=1.42 p—not significant
PBL of patients before removal of warts	32.35±5.41	45.41±7.46	t=3.49 p<0.01
PBL of patients one month after removal of warts	29.78±5.01	25.56±7.71	t=1.03 p—not significant
PBL of patients two months after removal of warts.	23.97±1.25	28.50±0.62	t=5.51 p<0.001
PBL of patients three months after removal of warts.	27.40±1.04	31.67±2.11	t=3.64 p<0.05

Comments

PBL of wart patients before the removal of their warts showed a significant rise of LAI when reacted with wart antigen. The NAI also showed a much higher value than that of the controls. On the other hand, when results of normal PBL were compared with patient's PBL, very little difference was obtained in the mean value after reacting with penile skin antigen, but a marked difference was detected after reacting the wart antigen with normal PBL and patient's PBL. This marked difference in the mean value in case of wart is due to the fact that the wart consists of two types of antigens—tumour antigen and viral antigen. In the past, in vitro assays of CMI to human wart antigen have shown inconclusive results in patients with a long history of wart infection.[5,6] Hence, it can be concluded that it is not only the viral antigen but also the associated wart antigen which are responsible for making the difference in the value of LAI reactivity.

After excision of warts, the results of LAI obtained by past workers,[4,7,8] differ markedly with our results. The reactivity of T-LAI does not disappear completely but gradually shows a higher mean value assessed at monthly intervals even when the patients are tumour free. In breast tumour,[4,7] melanoma,[8] tumours of the colon and rectum,[9] and laryngeal tumour[10] cases the reactivity of T-LAI disappeared rapidly after surgery.

We find that the reactivity does not completely disappear at the end of the first month, which is normally expected, and the further increase of T-LAI reactivity at the end of the second month when the patient's PBL have been reacted with penile skin extract and wart extract. The mean LAI after penile skin extract falls below that of the mean LAI after wart extract. At the end of the third month, the mean value of LAI, after penile skin extract and wart extract has boosted up and maintained almost parallel relationships with the second month.

Our results although obtained on a limited materials, indicate that the LAI assay can detect immunity against penile wart before and after their removal.

After removal of warts, some viral particles are still present in the local area which evoke antiviral immunological response. The anti-tumour immunological response most likely does not play any role in the absence of active lesion. It is also evident that the mean value of patient's LAI after wart antigen before removal (45.41 ± 7.46) is much higher than the mean LAI three months after excision of warts (31.67 ± 2.11). This difference is due to the absence of antigen associated with the wart tissue because of removal of the later.

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