

AN IN VIVO STUDY OF CELL-MEDIATED IMMUNITY IN HUMAN WARTS

Preliminary Results

J. Thivolet, M. R. Hegazy, J. Viac and Y. Chardonnet

*From the Laboratory of Immunology, Department of Dermatology, Hôpital Ed. Herriot, Lyon, France,
the Department of Pharmacology, Faculty of Medicine, Al-Azhar University, Cairo, Egypt, and
the Unité de Virologie INSERM, U 51, Lyon, France*

Abstract. Purified human papilloma virus (HPV) was used to study cell-mediated immunity (CMI) in patients with warts and in controls by means of the intradermal test (IDT). IDT was positive in 75 % of patients with past history of warts, in 56 % of patients presenting with warts, but in only 7 % of controls. Immunofluorescent antibody levels were initially low and increased after injection of HPV antigen in all groups.

Key words: Warts; Cellular immunity; Intradermal test; Immunofluorescent antibodies; HPV antigen

Many studies have been published on the immunology of human papilloma virus (HPV), most of them concerned with circulating antibodies. Only 20-50 % of patients with warts have circulating antibodies (1, 3, 7, 11). Humoral immunity probably plays a minor part in the cure of warts, which may depend on cell-mediated immune response (10, 7). The incidence of warts increased sharply after treatment with immunosuppressive drugs (12, 14) and in diseases which impair the immune system (9). Local inflammatory reaction often precedes spontaneous involution of warts (10, 13) and may be related to cell-mediated immunity (CMI).

In vitro tests of CMI on human warts were carried out (6). Skin tests in patients with warts have been reported previously (4, 5). However, total wart tissue extracts were used in these studies.

The present study is the first in vivo test on CMI using purified killed HPV.

MATERIAL AND METHODS

Collection of warts

Plantar and hand warts were collected by surgical excision, immediately frozen in liquid nitrogen and kept at -25°.

Purification of virus

Semi-purified virus was obtained from the lower band of Cesium chloride gradient (full particles) as prepared by Pass & Marcus (8).

The purity of the virus was tested by electron-microscopy, measurement of optical density at 260 and 280 nm, polyacrylamide gel electrophoresis (2), and Australia antigen. The virus was killed with 0.4 % formaldehyde (12 hours at 4°) dialysed against PBS and then diluted to contain about 3×10^{10} particles per 0.1 ml (5 μ g proteins).

Preparation of rabbit HPV antiserum

One rabbit was injected with 1 mg of purified HPV proteins emulsified in an equal volume of Freund's complete adjuvant and after 5 weeks by intravenous injection of 0.75 mg HPV proteins. The serum was collected 8 days after the last injection and was tested by immunodiffusion in agar gel (Ouchterlony) against HPV and in indirect IF on human wart sections.

Cellular immunity tests

Selection of patients. 1st group: patients with both a few and with numerous warts, have been studied in these preliminary tests. 2nd group: patients with a past history of warts which had regressed spontaneously or after treatment. 3rd group: control subjects with negative history of warts. The ages of the patients ranged between 1 and 80 years and the duration of warts from 3 months to many years.

Table I. IDT in patients with warts and in control subjects

	No. of cases	Positive IDT	%	P level
I. Present warts	34	19	56	I-III <0.001
II. Past warts	12	9	75	II-III <0.001
III. Control	56	4	7	

Table II. Circulating antibodies (IF test)

Group	No. of sera	Positive sera	%
I. Present warts			
Before IDT	28	2	7
After IDT	18	13	72
II. Past warts			
Before IDT	9	0	0
After IDT	6	2	33
III. Control			
Before IDT	30	1	3
After IDT	13	2	15

The study was carried out on plantar and hand warts including a few cases of *Condyloma accuminatum*.

Intradermal test. Patients were injected intradermally with 0.1 ml HPV suspension containing 5 µg virus proteins. Observations were made after 24, 48 and 72 hours. Erythema and induration after at least 24 hours were considered a positive result.

Humoral immunity tests

Sera of patients were collected before injection and 8 days subsequently. Indirect IF tests were performed as described by Genner (3). Frozen sections of warts that were positive to rabbit HPV antiserum were used to detect antibodies in patients' sera.

RESULTS

The results of IDT are shown in Table I. The percentage of positive ID reaction in patients with past history of warts or presenting with warts is significantly higher than in control subjects ($P < 0.001$). Patients of group II have the highest percentage of ID positive tests.

The results of indirect IF testing for antibodies are shown in Table II. The incidence of antibodies increased in all groups after IDT.

The cumulative findings of IDT and circulating antibodies are shown in Table III. Before IDT the

proportion of sera which showed wart virus specific antibodies was very low in all groups. After IDT the results showed a much higher incidence of circulating antibodies in subjects with positive IDT than in subjects with a negative test finding.

The relationship between the presence of a cell-bound immunity and the occurrence of different forms of warts has still not been studied.

DISCUSSION

The present results demonstrate clearly a specific cell-mediated immune reaction to HPV. Cellular immunity was more evident in groups I and II, which had been exposed to infection with the virus. The presence of CMI reaction in the control group may be explained by unnoticed exposure to infection. The highest proportion of positive ID reaction was noted in group II and was higher than that in group I, although more cases are needed to make a better interpretation. The results of in vivo study of CMI are evidently more specific and simpler than in vitro assays and more relevant to the problem of immunity in patients.

Preliminary results show a lower incidence of humoral immunity than CMI. The high percentage of positive IDT and the much lower incidence of circulating antibodies in the same group of patients before IDT suggest the much longer persistence of CMI than humoral immunity, which fades gradually. The incidence of antibodies was much higher in patients with positive IDT only after injection, than in the controls (Table III). These results may be interpreted as a booster effect.

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Table III. Correlation between IDT and circulating antibodies in all groups

ID reaction	Before IDT (no. of sera)			After IDT (no. of sera)		
	+	Total	%	+	Total	%
+	2	27	8	15	18	84
-	5	45	11	4	19	21

^a $P < 0.001$.

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J. Thivolet, M.D.
Department of Dermatology
Hôpital Ed. Herriot
Place d'Arsonval
F-69374 Lyon Cedex 2
France