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An Antigen from Human Oral Cancer an Attempt for Isolation

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An antigen from human cancer for the first time was demonstrated by Gold and Freedman in 1965 in the Colonic Carcinoma.[1] Since then many attempts are being made to isolate specific antigens from various types of malignancies [2,3,4,5,6,7,]. These tumour associated antigens are accepted parameters of prognostic significance and are under way of evaluation in the field of immunotherapy. From the epidemiology of various cancers in Regional Cancer Centre at Trivandrum, it has been shown that 30% of total admissions in the Centre comes from Oral Cancers. Considering this magnitude of the problem, we are working on the immunological aspects of oral cancer. Here the successful isolation of a tumour associated antigen from human oral cancer is described.

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Material and Methods

Biopsy:

Tha specimens were collected from the operated tissues of oral cancers. Large number of patients were included in this work. But biopsies mainly constituted from a single type of malignancy - cauliflower like growth of squamous cell carcinoma.

Homogenisation:

The pooled specimens were stored at -70° C and a homogenate was prepared modifying the method of Gold and Freedman [1] Normal oral mucosa was also pooled.

Biopsy and all laboratory procedures were carried out under strict aseptie precautions. The antigen was lyophilised for storage. It

was filtered and protein estimation done by Lowry's method. Frequent bacteriological tests were done to assure sterility.

Immunisation:

Rabbits 6 months old were used to raise the Antisera. Homogenised Oral Cancer Crude Antigen was emulsified into Freund's Complete adjuvant and 6 injections were given at 3 Wk. intervals. Part of the antisera was completely absorbed into normal oral mucosa.

Gel Filtration

Sephadex G 100 was used for the fractionation procedure using PBS of p H 7. 4 Column Chromatography was done into both Cancer homogenate and normal oral mucosal homogenate. The different protein fractions were separately collected and lyophilised for further immunological testing.

Ouchterlony preciptation:

In the immuno diffusion plate different types of cross reactivity were tested.

SDS PAGE

SDS Polyacrylamida Gel Electrophoresis using Phosphate Buffer of pH 7.4 was done with Crude Oral Antigen and specific Antigen.

Observations

Immunodiffusion plates showed 3 distinct lines of precipitation between the Crude Oral Antigen and the unabsorbed sera. Whereas only one line appeared between the same antigen when tested with absorbed antiserum (Fig. 1).

Immuno electrophoresis showed different crescents of precipitation with the unabsorbed Serum, whereas there was only one towards as the absorbed through (Fig. 2).

The protein concentration of the homogenate was standardised to be 1 mg/ml. The column chromatography showed 3 distinct peaks with Crude Antigen whereas only 2 peaks were observed with Normal mucosal homogenate. The first peak in the column with

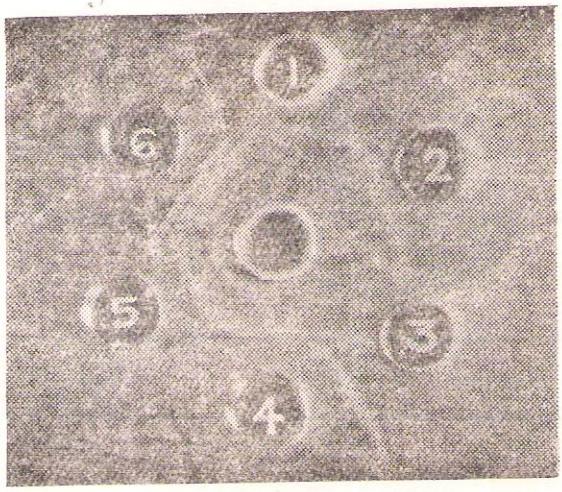

FIG. 1. **Immuno Diffusion Plate**

Central Well contains Crude Oral Cancer Antigen. Wells 1, 2 & 3 = Absorbed Antiserum. 4, 5 & 6 = Unabsorbed Antiserum.

Note three lines of precipitation on the unabsorbed side and single line on the absorbed side.

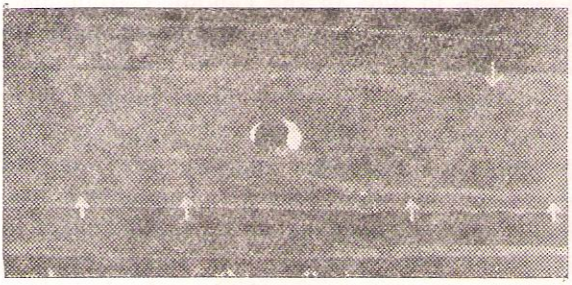

FIG. 2. **Immuno Electrophoresis.**

Central Well = Crude Oral Cancer Antingen.

Note 4 bands of precipitation towards the through which contains unabsorbed antiserum and single band of precipitation towards the absorbed antiserum.

Crude homogenate appeared immediately after the void volume and it had a shouldering.

There was no precipitation between the other embryonal Antigens and the oral cancer Antisera, as there was no cross reaction between stomodial Ag and CEA (Fig. 3). SDS PAGE showed 4 different bands with Crude Oral cancer antigen whereas the immediate portion of the shoulder from the first fraction of Gel Filtration (Sephadex G 100) was found moving as a single band.

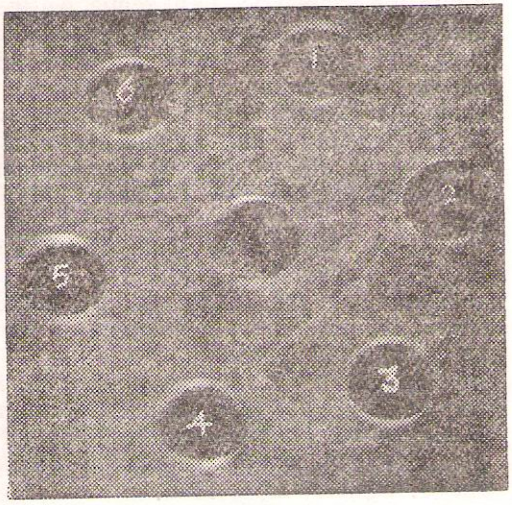

FIG. 3. **Immuno Diffusion Plate showing specificity of Ag.**
Central Well = Absorbed Serum.
Wells 3 & 6 = Fraction No. I from Crude Oral Cancer Antigen.
Wells 1 & 4 = Stomdial Antigen
wells 2 & 5 = Carcino Embryonic Antigen.
Only Fraction No. I (Specific Ag) gives the precipitation line.

Discussion

During malignant transformation, cell surfaces acquire new antigens. After the pioneering work of Gold and Freedman in 1965 on CEA, a list of other tumour associated Ags were demonstrated in various malignencies. Specificity is the criterion are antigen has to possess immunologically to prove its use fulness in early detection, prognostic evaluation and immunotherapy.

This paper presents the first successful isolation of a tumour associated antigen from human Oral Cancer. The Column Chromatography after Sephadex G 100 shows a shouldering effect of the first peak. This could be due to combination of two fractions together getting eluted. Further gel filtration using Sephadex G 200 is in progress in the laboratory.

Specific cross reactivity tests with other embryonal antigens in the Ouchterlony plate excludes this specific fraction, isolated now to be an embryonal one. However more works in this line have to be continued.

Delayed type cutaneous hypersensitivity tests and in-vitro immunological parameters like Macrophage migration inhibition test (MMI), Leukocyte adherence inhibition Test (LAI) Blast transformation and passive haemagglutination inhition tests etc are all encouraging. An attempt to culture the sensitised lymphocytes from Oral Cancer patients is also on progress.

Immunotherapy has been tried using the lung cancer associated antigen from abroad.[12] Our laboratory works so far, indicate to the specificity of the Oral Cancer antigen and it is attempted to establish specific Immunotherapy trial in oral cancer patients. The molecule is now being purified.

Standardisation of RIA of this OCA is now being attempted in our laboratory.

Summary

A specific fraction of the tumour tissue has been isolated from human oral cancers. It does not share cross reactivity with the available embryonal antigen so far tested. The cell mediated immunity as evidenced by DTH reactions and the invitro immunological assessments show that this particular antigen can be used for specific immunotherapy.

Further purification of the antigen is in progress.

Acknowledgment

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