

# Thymus derived lymphocytes (T cells) in patients with genital warts

K C MOHANTY\* AND R B ROY†

*From the Departments of Genitourinary Medicine, \*St Luke's Hospital, Bradford BD5 0NA and †Royal Victoria Hospital, Bournemouth BH7 6JF*

**SUMMARY** Thymus derived lymphocytes (T cells) were counted in the peripheral blood of 30 patients with genital warts and in 20 healthy controls. The control group was made up of 10 healthy patients with no history of warts and 10 who had been cured of warts for at least 12 months. We found that patients with genital warts had a significantly lower number of T cells despite an adequate number of circulating lymphocytes in the peripheral blood. We therefore suggest that a functional defect of lymphocytes ("dyslymphocytosis") could be the cause of genital warts either in their primary or recurrent form. These abnormal lymphocytes return to their normal function after the disappearance of genital warts.

## Introduction

Genital warts are one of the most common conditions (28 176 cases reported in 1981 in England) seen in genitourinary clinics in the United Kingdom. Although cutaneous warts are mostly seen in dermatological clinics, it is not uncommon to see them in genitourinary clinics. The viral aetiology and existence of specific immune responses have been well established.<sup>1</sup> Various studies have shown that cell mediated immune responses measured *in vitro* and *in vivo* are diminished in individuals with cutaneous warts.<sup>2,3</sup> It is interesting to note that the immune response of patients with cutaneous warts is different from that of those with genital warts.<sup>4</sup> We cannot find any report of a previous study that used the rosette assay to measure the numbers of T cells in the peripheral blood of patients with genital warts. The lymphocytes that bind *in vitro* to sheep erythrocytes in a rosette formation are thymus derived, and are called T cells or E rosettes. It has also been reported that persistent viral infection (more than one year) might contribute to depression of T cells.<sup>3,5</sup> This study was therefore aimed at determining by rosette assay the numbers of T cells in patients who had had genital warts for more than 12 months, and their significance in the pathogenesis of primary or recurrent genital warts.

Address for reprints: Dr K C Mohanty, Consultant in Genitourinary Medicine, St Luke's Hospital, Bradford BD5 0NA

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## Materials and methods

Blood samples were taken from 30 patients who gave a history of genital warts of at least twelve months' duration. They did not give a history of cutaneous warts or any other recent viral infection. The mean age of this group was 22 (range 17-30) years. Blood samples were also taken from 20 control subjects, of whom 10 had a history of genital warts but had been free of them for at least a year. The other group of 10 controls had no history of genital warts. Neither of the sub-groups of control subjects had warts on examination. Their mean age was 25 (range 18-35) years. The blood samples were tested for total white cell and lymphocyte counts per litre of peripheral blood. Percentages of E rosettes were recorded by the rosette assay.

### ROSETTE ASSAY

Peripheral blood from patients and controls was collected in heparin without preservative. Mononuclear cells were isolated by Ficoll-Trisil gradient sedimentation, washed three times with buffered Eagle's medium, and resuspended in this medium supplemented with 2% heat inactivated fetal calf serum at a concentration of  $2 \times 10^6/l$ . Four 20  $\mu$ l drops of indicator red cells and four 20  $\mu$ l drops of lymphocytes were mixed in a plastic preparation tube (50  $\times$  4 mm), incubated for 15 minutes at 37°C, and then centrifuged at  $200 \times g$  for five minutes at room temperature. The pellet was then immediately placed in ice and kept at 4°C for one hour. A 0.2 ml volume

of this preparation was loaded into the cytocentrifuge wells with siliconised pasteur pipettes and centrifuged at  $20 \times g$  for five minutes to make four slides in each case. Finally the slides were stained by the Romonowsky technique. Rosettes were counted as lymphocytes binding three or more indicator cells; aggregates of more than four lymphoid cells were not counted. Rosetted lymphocytes were counted against the background of evenly distributed indicator cells; peripheral areas of the smear being avoided because of clumping caused by the preparation technique.

We did not perform a suspension method of detecting the E rosettes as both cytocentrifuge and suspension methods gave results within 5% variation in the same laboratory.<sup>7</sup> The cytocentrifuge method is used routinely and gives a normal mean (SD) of 70% (10%) of E rosettes. The reproducibility of the technique for repeated analysis for healthy people is not greater than 10%. We repeated this test in five control subjects for confirmation.

## Results

The figure shows that the total white cell and total lymphocyte counts per litre and differential white cell count were within normal ranges both in patients and control groups. The morphology of lymphocytes on microscopical examination was normal. The total number of T cells  $\times 10^6/l$  of peripheral blood in patients with genital warts was calculated from their percentage and was found to vary from  $580 \times 10^6/l$  to  $1691 \times 10^6/l$ , whereas the number of rosettes in healthy subjects (with either no history of genital warts or no genital warts in the past year) was within the range of  $810 \times 10^6/l$  to  $2920 \times 10^6/l$ .

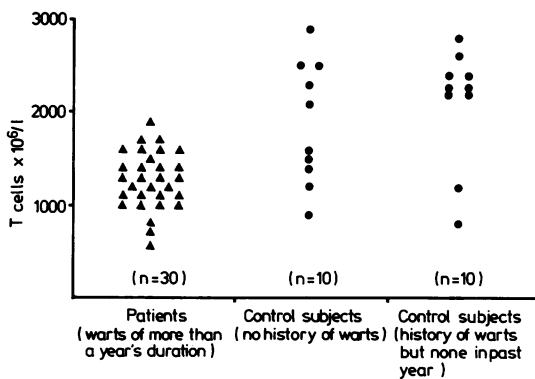


FIGURE Numbers of T cells in patients with warts and in control subjects.

Comparison of the two control groups using the Mann-Whitney U test gave  $U = 42.5$  in sample sizes of 10 and 10. This value lies well within the range of

random fluctuation, and we concluded that there was no significant difference between the median responses of the two control groups. Combining them was therefore acceptable, and this gave a pooled control group of 20 people against which we may compare the study group of 30 patients. The Mann Whitney U test then gave  $U = 144$  in sample sizes of 30 and 20. This value was significant at the 0.1% level ( $p < 0.001$ ) and provided powerful evidence that there was a shift in median response between the two groups.

## Discussion

Humoral immunity is thought to play little part in acquisition, regression, or recurrence of warts. The appearance of complement fixing IgG antibodies before or during treatment, however, could lead to their rapid resolution.<sup>3</sup> It is well established that cellular immunity plays a major role in the control of viral infections. Various studies using different methods such as lymphocyte transformation,<sup>5</sup> leucocyte migration inhibition,<sup>5</sup> response to purified protein derivative and phytohaemagglutinin<sup>6</sup> have shown an appreciable cell mediated immune deficiency in patients with recurrent and chronic cutaneous warts. This study shows a significant ( $p < 0.001$ ) reduction in numbers of T cells in patients who had been suffering from genital warts for at least 12 months. These findings are similar to those in other published papers which reported a deficiency in rosette forming capacity in patients with cutaneous warts.<sup>3</sup> Secondly, when the numbers of T cells per unit volume of peripheral blood in both control groups were compared by the Mann-Whitney U test there was no appreciable difference between the two groups. This indicates a return of adequate cell mediated immunity, which was probably depressed during the presence of warts in the 10 control subjects who at one time had had genital warts. It is known that viral particles can be identified in warts for several months, and these continue to depress T cells during the life span of cutaneous warts.<sup>3</sup> This hypothesis is probably also true in cases of genital warts.

Another interesting aspect of this study is that all the subjects, both patients and members of the control groups, had total lymphocyte counts within the normal range ( $15 \times 10^9/l$  to  $4 \times 10^9/l$ ) despite the significant differences between their abilities to form rosettes. The lymphocytes that look normal but have inadequate ability to form rosettes, which are found in patients with genital warts, therefore have a functional defect or dysfunction that could be termed "dyslymphocytosis". We believe that this dyslymphocytosis plays a major part in the patho-

genesis of genital warts, either in their primary or recurrent form.

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