An immunohistological study of spontaneous regression of condylomata acuminata

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Abstract

An immunohistological study of four men whose perianal warts were undergoing spontaneous regression was undertaken, and the results compared with those obtained from non-regressing condylomata from six men. $CD4^+$ and $CD8^+$ cells were noted in the stroma of each wart, but there was no clear difference in the density of the infiltrate between regressing and non-regressing warts. Natural killer cells ($CD16^+$ and $CD57^+$) were only noted in the stroma and epidermis of regressing warts. Possible immunological mechanisms of regression of condylomata acuminata are discussed.

It is common clinical experience that anogenital warts in immunocompetent individuals eventually undergo spontaneous regression. However, the immunological mechanisms involved in this process are largely unknown. As condylomata acuminata are often florid and persistent in individuals with deficient cellular immunity[1] it is likely that cellular immune processes are important in the resolution of wart virus infection. We undertook an immunohistological study of four individuals whose perianal warts were undergoing spontaneous regression and compared the findings with those in stable condylomata.

Materials and methods

The study was approved by the relevant ethical committee of the Lothian Health Board.

PATIENTS

During an 18 month period spontaneous regression of warts was noted in four men (cases 1–4). These men had agreed to participate in a double-blind trial of α-interferon in the treatment of anogenital warts and each had received a subcutaneous injection of placebo three times per week for 3 weeks. They had not been treated previously for warts. Prior to the first injection, scissor excision of representative lesions was undertaken. Although clinical signs of regression were inapparent at the patients' initial attendance, complete regression of the condylomata occurred within 4 weeks.

The immunohistological findings in regressing warts were compared with those in perianal warts removed from six previously untreated men (cases 5–10) whose lesions did not regress within six months of their initial attendance and biopsies. Podophyllin and cryotherapy were used to treat these individuals after the biopsy sites had healed.

Other sexually transmitted infections were excluded by the appropriate microbiological investigations. After obtaining informed consent and after counselling, serum from each patient was examined by a commercial ELISA method (Abbott) for antibodies against the human immunodeficiency virus (HIV); in each case negative results were obtained.

IMMUNOHISTOLOGICAL METHODS

The warts were snap-frozen in liquid nitrogen and stored at −70°C until required. Cryostat sections (4 μm) were cut and stained for cell surface antigens using a panel of monoclonal antibodies (table 1) in an

Table 1 Monoclonal antibodies used in the study

CD	*Antibody*	*Reactivity*	*Source*
19	CD19	B lymphocytes	Dako
22	M708	B lymphocytes	Dako
3	Leu 4	T lymphocytes	Becton Dickinson
4	Leu 3a	Helper/inducer T cells	Becton Dickinson
8	Leu 2a	Suppressor/cytotoxic T cells	Becton Dickinson
25	IL-2R	Interleukin-2 receptors	Dako
16	Leu 11b	FcRIII, natural killer cells	Becton Dickinson
57	Leu 7	HNK-1, natural killer cells	Becton Dickinson
1a	Leu 6	Langerhans' cells	Becton Dickinson
	DA6. 231	HLA-D framework	Dr K Guy
	RFDR-1	HLA-DR	Scottish Antibody Production Unit
	L243	HLA-DR	American Type Culture Collection
	B7/21	HLA-DP	Dr K Guy
	Leu 10	HLA-DQ	Becton Dickinson

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indirect immunoperoxidase method.[2] Several sections were stained with haematoxylin and eosin and examined by one of us (SF) who was unaware of the clinical findings.

Cells in the wart stroma were counted using an eyepiece graticule with a 10 × 10 grid: the sections were examined with a ×40 objective and a ×10 eyepiece giving a grid edge 0·283 mm long, and of area 0·08 mm^2. A minimum of three high power fields were counted by two independent observers unaware of the clinical findings at the time of counting. Interobserver variation was <5% of the mean counts. The distribution of cells per unit area was uniform, interfield variation being <5%.

Results

The stroma in each case was mildly oedematous, and moderate fibrosis was noted in four warts (cases 3, 6, 7, 8). A mild to moderate stromal infiltrate of lymphocytes was noted and occasional lymphocytes were found in the epidermis.

Table 2 summarises the immunohistological findings. B lymphocytes were noted only in the stroma of the condylomata from case 9. There was marked variation in the mean number of T lymphocytes (CD3$^+$) per unit area of stroma from wart to wart. In each case CD4$^+$ cells predominated, but the proportion of CD3$^+$ cells that expressed CD8 varied considerably from case to case. CD8$^+$ T cells were noted only occasionally within the epidermis.

The T' index (mean number of three fields of cells expressing CD25 (interleukin-2 receptors)/mean number of cells in the same fields reacting with CD3) was variable and there was no clear difference between the proportion of CD25$^+$ cells in warts that regressed within four weeks of biopsy and those that remained unchanged.

A feature of the warts removed from cases 1–4 was the presence in the stroma or epidermis of cells that reacted with either CD16 (Leu 11b) or CD57 (Leu 7) or both (table 2).

CD1$^+$ epidermal Langerhans' cells and small numbers of CD1$^+$ stromal dendritic cells were noted in every case (table 2). There was no significant difference in their density in the epidermis between warts that were about to regress and those that were not. In each case, epidermal Langerhans' cells expressed HLA-DR. HLA-DP and HLA-DQ were expressed on the surfaces of these cells in each of the nine warts studied; fewer cells, however, expressed HLA-DQ than HLA-DR and -DP (data not shown).

Focal staining of keratinocytes particularly in the basal areas of the epidermis was noted with the monoclonal antibody DA6.231, which is broadly reactive with MHC class II antigens, as well as with the antibodies reactive with HLA-DR, in cases 1, 2, 4, 5, 7, 9, 10. Material for testing was not available from case 3. Keratinocytes expressing HLA-DP and HLA-DQ were not detected. HLA-DR was expressed on the surface of many of the mononuclear cells infiltrating the dermis.

Discussion

Although spontaneous regression of anogenital warts is well recognised, the immunohistology of regressing condylomata acuminata has not been described previously.

In regressing plane warts there is dilatation of the small blood vessels of the upper dermis, marked infiltration of the dermis with mononuclear cells, and epidermal degenerative changes.[3] A detailed immunohistological study has shown that the dermal infiltrate contains CD4$^+$ and CD8$^+$ cells, the former cell type predominating.[4] The finding of T cells in apposition to Langerhans' cells in the epidermis and the congregation of lymphocytes or macrophages around keratinocytes in spongiotic epidermis suggests that cell mediated immunity against virus-infected keratinocytes occurs during plane wart regression.[4]

As anogenital warts can be extensive in individuals with defects of cellular immunity,[5] it is likely that

Table 2 Immunohistological findings in perianal warts

Case number	Age (yr) (sexual orient.)	Duration of warts before biopsy (months)	Number of cells per unit area* in stroma reacting with monoclonal antibodies: CD19	CD22	CD3	CD4	CD8	CD25
1	21 (het)	1·5	0	0	221	210	90	9
2	55 (het)	3	0	0	143	112	50	15
3	21 (homo)	8	0	0	33	31	5	ND
4	20 (het)	8	0	0	204	144	102	18
5	23 (het)	5	0	0	373	294	58	50
6	21 (het)	5	0	0	74	76	20	3
7	24 (het)	3	0	0	18	25	5	0
8	20 (homo)	3	0	0	32	34	9	0
9	24 (het)	1	0	2	275	251	91	33
10	20 (het)	3	0	0	199	198	17	35

het = heterosexual; homo = homosexual; ND = not determined; *Mean number of three areas; − = negative; ± = occasional cell.

cell-mediated immune responses are also important in resolution of anogenital human papillomavirus (HPV) infection. Our observations suggest several mechanisms, all of which may play a role in the regression of condylomata acuminata.

Langerhans' cells can present antigen to T lymphocytes[11] and as diminished numbers and morphological abnormalities of the epidermal Langerhans' cells and dermal dendritic cells in condylomata have been reported,[12,13] it has been suggested that their depletion may result in a diminished host defence against viral infection and explain the persistent nature of anogenital warts. We, however, failed to note any difference in the density of epidermal Langerhans' cells between condylomata that were about to regress and those that were not.

Keratinocytes of normal skin do not express HLA-DR. In certain diseases such as lichen planus, however, keratinocytes overlying a lymphocytic infiltrate can be shown to express HLA-DR.[6] This may be the effect of γ-interferon released from activated T cells, many of which also express the CD25 antigen (interleukin-2 receptors) early during activation.[7] Although CD25 antigen is also expressed weakly by activated B cells and by some myeloid cell lines, we did not find B cells (except for small numbers in case 9) and the cells staining appeared to be lymphocytes, and therefore presumably T cells. We did not, however, find a significant difference in the T' index between warts undergoing regression and those not. As HLA-DR expression on keratinocytes is associated with epidermotropism for $CD8^+$ cells,[8] such cytotoxic T cells may play a role in the resolution of HPV infection. Satellite cell necrosis, a hallmark of cytotoxicity where a single dyskeratotic keratinocyte is surrounded by lymphoid cells, has been noted during the regression of plane warts.[4] Although we did not observe satellite cell necrosis, we have shown the presence of $HLA\text{-}DR^+$ keratinocytes and $CD8^+$ cells in the epidermis of condylomata, and a similar mechanism for regression of condylomata acuminata cannot be entirely discarded.

The role of natural killer cells in resolution of HPV infection is uncertain. External genital warts have been reported in a girl with Fanconi's anaemia and a selective defect of natural killer cell function.[9] Tay *et al*[10] noted a moderate infiltrate of such cells in the stroma of the cervix from five of six women with histological evidence of HPV infection at this site; natural killer cells were found only in small numbers in the normal cervix. As we only found these cells in the stroma and/or epidermis of each of the lesions that were about to undergo spontaneous regression, they may be determinants of regression.

Number of cells per unit area in stroma (presence of cells in epidermis) reacting with natural killer cell monoclonal antibodies:*		*Number of cells per unit area* of epidermis reacting with monoclonal antibody:*
CD16	*CD57*	*CD1a*
25 (+)	3 (−)	8
0 (±)	0 (−)	10
15 (±)	10 (±)	ND
8 (±)	6 (−)	8
0 (−)	0 (−)	6
0 (−)	0 (−)	8
0 (−)	0 (−)	9
0 (−)	0 (−)	6
6 (−)	0 (−)	8
0 (−)	0 (−)	15

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