Expression of major histocompatibility class II antigens by Langerhans' cells in cervical intraepithelial neoplasia

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SUMMARY Cervical biopsy samples from 67 patients who had various grades of cervical intraepithelial neoplasia (CIN) or who showed evidence, in the form of koilocytosis, of human papillomavirus (HPV) infection of the uterine cervix, and from 10 women with normal cervixes were examined. Cryostat sections from the biopsy samples were stained using monoclonal antibodies to T6, a Langerhans' cell marker, and to major histocompatibility complex (MHC) class II antigens (HLA-DP, DQ, and DR). Epithelial Langerhans' cells were reduced in number and showed changed morphology and distribution in koilocytic lesions and in all grades of CIN ($p < 0.01$) except CIN I. HLA-DR expression by Langerhans' cells was significantly increased in koilocytic lesions and in CIN grades I and II ($p < 0.05$); HLA-DQ expression was significantly increased in all grades of CIN ($p < 0.05$) with the increase being most pronounced in CIN I ($p < 0.01$). Columnar epithelium expressed MHC class II antigens in all samples tested and squamous epithelium in four of 29 cases of CIN III.

These findings support the view that there is a localised disturbance of immune function in both neoplastic cervical epithelium and that infected with papillomavirus.

The association between human papillomavirus (HPV) infection and cervical intraepithelial neoplasia (CIN) has been well documented, and an increasing body of evidence suggests that this association is causal rather than casual.¹ Other factors probably also have a role, and recent reports of HPV DNA isolation from apparently normal cervical epithelium^{2,3} would support the hypothesis that the disease develops by a multistep process.

The nature of an effective immune response to papillomavirus infections is not well understood, although cell-mediated immunity is thought to be more important than humoral immunity.⁴ A deficient immune response to HPV may permit the development of intraepithelial neoplasia, and a reduction in the number of antigen presenting cells has been shown in both papillomavirus infected⁵⁻⁸ and neoplastic cervical epithelium.⁸

The predominant antigen presenting cell in squamous cervical epithelium is the Langerhans' cell, a dendritic cell which may be identified by the use of

monoclonal antibodies to the common thymocyte antigen T6,⁹ or to major histocompatibility complex (MHC) class II antigens.¹⁰ The ability of the Langerhans' cell to present foreign antigen to T lymphocytes depends on the expression of surface MHC class II antigens.¹¹ The level of MHC class II antigen expression by Langerhans' cells is known to vary¹² and may reflect the state of "activation" of the cell. The recent widespread availability of monoclonal antibodies now facilitates study not only of the framework determinants common to all MHC class II antigens, but also the non-polymorphic products of the individual known class II loci HLA-DP, DQ, and DR.¹³⁻¹⁵

In this study we attempt to relate the number, distribution, and morphology of Langerhans' cells and their expression of MHC class II antigens (including the products of HLA-DP, DQ, and DR) to the presence of HPV infection, in the form of koilocytosis, and of CIN grades I–III.

Patients and methods

Sixty seven patients (age range 19–45 years) referred to the Lothian Area colposcopy clinic with a history of

abnormal cervical cytology were studied. Seven women (aged 20–38) who had been referred to the colposcopy clinic for other reasons (four women on account of clinical concern about the appearance of the cervix and three women because of post-coital bleeding) and who had no cytological, colposcopic, or histological evidence of CIN were included as controls. Three women (aged 33–41) undergoing total abdominal hysterectomy for benign conditions, with a history of normal cervical cytology, were included as further controls.

A cervical biopsy sample was taken from the transformation zone of each patient and control. All the samples from the patients came from an area of colposcopic abnormality while those from the seven colposcopy clinic controls came from an area of suspected colposcopic abnormality within which no histological evidence of CIN or HPV infection was subsequently found. Those from control patients undergoing hysterectomy were taken within 15 minutes of the uterus being removed from the patient. The specimens were snap frozen on dry ice and stored at -80°C until processed further. An 8 μm cryostat section was cut from the centre of each and prepared for routine histopathological examination with a conventional haematoxylin and eosin stain. CIN was classified according to recognised criteria.¹⁶ Koilocytes (balloon shaped cells with a perinuclear halo) were considered to be diagnostic of HPV infection.¹⁷

Serial cryostat sections (8 μm in thickness) were prepared, mounted on poly L-lysine coated slides, fixed in acetone at 4°C for 10 minutes, and air dried. Sections were then stained using an indirect immunoperoxidase technique adapted from the method of Carr *et al.*¹⁸ Briefly, sections were incubated in 20% normal rabbit serum in 0.001M Tris-buffered saline (TBS), pH 7.6, for 20 minutes. Excess serum was shaken off and sections then incubated for 45 minutes with monoclonal antibody (table 1) at room temperature. DAKO T6 is ascitic fluid,¹⁹ and all other monoclonal antibodies are culture supernatants.^{13–15 20} The optimal dilution of antibody made in 2% normal rabbit serum was determined by titration. Negative

controls were prepared by substituting 2% normal rabbit serum for monoclonal antibody. Sections were then washed in 2% normal rabbit serum and incubated with a rabbit antiserum to mouse immunoglobulins conjugated with horseradish peroxidase (Dakopatts) for 15 minutes. The enzyme reaction was developed using diaminobenzidine (Sigma, Poole, Dorset). Sections were counterstained with Mayer's haemalum, washed in tap water, and mounted in DPX after dehydration.

QUANTIFICATION OF LANGERHANS' CELLS

Stained sections were photographed and the surface area of squamous epithelium calculated using tracing paper. Cells possessing at least two dendrites attached to a cell body and staining positively with DAKO T6 were classified as Langerhans' cells and counts expressed per mm^2 of epithelium, as this has been found to be the most reliable method of enumerating Langerhans' cells.²¹

The significance of the difference between each group and the control group was calculated using the Mann-Whitney U test.

Results

DISTRIBUTION AND NUMBERS OF LANGERHANS' CELLS

In specimens taken from normal cervical transformation zones Langerhans' cells were found predominantly in the lower half of the epithelium (fig 1). The slender dendritic processes stained strongly with DAKO T6 and appeared to link adjacent Langerhans' cells. No significant differences in Langerhans' cell morphology, distribution, number, or MHC class II expression were seen if biopsy samples from the two groups of normal controls were compared. In specimens taken from areas of CIN (fig 2) Langerhans' cells were distributed throughout the entire thickness of the epithelium and their dendrites were much shorter and less numerous. Table 2 shows that T6 positive Langerhans' cells were significantly reduced in number ($p < 0.01$) in all abnormal groups except CIN I.

Table 1 *Origin and specificity of monoclonal antibodies*

Monoclonal antibody	Working dilution	Origin	Specificity
DAKO-T6	1/20	Dakopatts ¹⁹	Langerhans' cells, common thymocytes
DA6.231	1/10	K Guy ²⁰	HLA-DP, HLA-DQ, HLA-DR
B7/21	Neat	I Trowbridge ¹³	HLA-DP
TU22	Neat	A Ziegler ¹⁴	HLA-DQ
L243	1/20	American Type Culture Collections ¹⁵	HLA-DR

EXPRESSION OF MHC CLASS II ANTIGENS BY LANGERHANS' CELLS

When the monoclonal antibody DA6.231 was used the number of positively staining dendritic cells closely paralleled the number of T6 positive cells, although it can be seen from tables 2 and 3 that T6 positive dendritic cells always exceeded DA6.231 positive dendritic cells in number. Some Langerhans' cells were therefore not expressing class II antigens, or were expressing these antigens at undetectable levels. In each specimen there were some DA6.231 positive, T6

Fig 1 *DAKO-T6 staining of normal cervical transformation zone showing dendritic Langerhans' cells in lower half of epithelium.*

negative, non-dendritic cells, and these were assumed to be activated T lymphocytes. This was confirmed in some cases (results not shown) by the use of monoclonal antibodies to T cell markers.

Table 3 shows the pattern of expression of HLA-DP, HLA-DQ, and HLA-DR by Langerhans' cells. A significantly higher percentage of Langerhans' cells in koilocytic lesions and in CIN grades I and II expressed

HLA-DR when a comparison was made with HLA-DR expression by Langerhans' cells in normal cervical epithelium ($p < 0.05$). No increase in HLA-DQ expression by Langerhans' cells was seen in koilocytic lesions, but significantly more Langerhans' cells were HLA-DQ positive in all grades of CIN than in normal epithelium (CIN I $p < 0.01$; CIN II and III $p < 0.05$). Significantly more Langerhans' cells were HLA-DP

Fig 2 *DAKO-T6 staining of sample of CIN I, showing Langerhans' cells throughout epithelial thickness.*

Table 2 Number of dendritic cells positive for T6 and DA6.231/mm² sectional area epithelium

No of cells/mm ²	Normal (n = 9)		Koilocytosis only (n = 6)		CIN I (n = 21)		CIN II (n = 10)		CIN III (n = 19)	
	T6 DA6.231		T6 DA6.231		T6 DA6.231		T6 DA6.231		T6 DA6.231	
Mean (SEM)	57 (12)	55 (10)	21 (5)	15 (4)	36 (6)	35 (6)	24 (6)	22 (8)	11 (3)	7 (4)

positive in CIN III than in normal epithelium ($p < 0.01$) but the increases seen in CIN I and II were not significant. There was a reduction in HLA-DP expression by Langerhans' cells in koilocytic lesions, which reached significance ($p < 0.05$).

Table 4 shows a comparison of Langerhans' cell numbers in users and non-users of the contraceptive pill. The number of Langerhans' cells was higher in users than in non-users in normal and in all the abnormal groups, but the difference was significant only in CIN I ($p < 0.05$).

In three of 10 specimens from normal transformation zones and 16 of 67 specimens from abnormal transformation zones a portion of columnar epithelium was included in the sections. In all 19 cases this epithelium stained positively with DA6.231 (fig 3) but not with the other monoclonal antibodies.

Normal squamous epithelium from the transformation zone and specimens of squamous epithelium from areas of koilocytosis only, and of CIN I and II, were consistently negative with all monoclonal antibodies used. In four of 29 specimens of CIN III, positive staining of squamous epithelium was seen using DA6.231, with parabasal cells staining particularly strongly. Staining with other monoclonal antibodies was always negative. These four cases were excluded when DA6.231 positive Langerhans' cells were being counted, as it was not possible to distinguish class II positive Langerhans' cells from positively staining squamous epithelium.

Discussion

DISTRIBUTION AND NUMBERS OF LANGERHANS' CELLS

We have shown that Langerhans' cells are reduced in number in HPV lesions and in CIN grades II and III, and that their distribution and morphology are changed within abnormal cervical epithelium. This is consistent with a recent report from Tay *et al.*,⁸ although earlier studies^{5,7} reported a decrease in Langerhans' cell numbers in HPV lesions and an increase in numbers in CIN. It has been postulated that the papillomavirus exerts a direct cytotoxic effect on Langerhans' cells,⁵ and the loss of dendrites observed may be a reflection of this. Work is in progress using a technique to label Langerhans' cells and papillomaviral antigen simultaneously to investigate this. Interestingly, there was abnormal distribution of Langerhans' cells throughout dysplastic epithelium and there may be an association between this and the pattern of HPV infection of squamous epithelium. HPV DNA can be detected by *in situ* hybridisation in the parabasal layers but viral antigens are normally detected only in the more superficial layers of the epithelium.²² Perhaps the antigen positive cells attract Langerhans' cells towards the surface away from their normal parabasal position. Once at the site of infection Langerhans' cells may be able to present HPV antigens locally to T lymphocytes or, having been activated by the local

Table 3 Percentage of Langerhans' cells (T6 positive dendritic cells) expressing MHC class II antigens

	DA6.231 *Mean (range) %	DR Mean (range) %	DP Mean (range) %	DQ Mean (range) %
Normal (n = 7)	92 (81-98)	57 (41-73)	35 (11-56)	22 (7-52)
Koilocytosis only (n = 4)	73 (63-95)	94† (64-117)	11† (4-26)	20 (9-46)
CIN I (n = 14)	93 (61-99)	72† (52-93)	47 (21-82)	65‡ (10-91)
CIN II (n = 8)	88 (65-100)	81† (61-94)	46 (27-69)	59† (8-73)
CIN III (n = 11)	72 (53-92)	46 (33-78)	85† (43-98)	57† (4-81)

*The percentage of Langerhans' cells staining positively with each anti-MHC class II monoclonal antibody was calculated for individual specimens before obtaining a mean value.

Significance of differences compared with normal group; † $p < 0.05$; ‡ $p < 0.01$ (Mann-Whitney U test)

Table 4 No. of dendritic cells/mm² in samples from users and non-users of contraceptive pill

No. of cells/mm ²	Users	Non-users
Normal epithelium (Mean (SEM))	82 (29)	49 (14)
Koilocytosis only (Mean (SEM))	23 (13)	21 (5)
CIN I (Mean (SEM))	49 (15)	26 (9)
CIN II (Mean (SEM))	29 (6)	25 (10)
CIN III (Mean (SEM))	13 (6)	9 (4)

viral infection, may leave the site and present HPV antigens in draining lymph nodes.

MHC CLASS II EXPRESSION BY LANGERHANS' CELLS

Table 2 shows that not all T6 positive Langerhans' cells were stained with the pan-class II marker DA6.231. This has been reported previously^{6,8,23} using other monoclonal antibodies to class II antigens, and may reflect the variable expression of these antigens by Langerhans' cells. HLA-DR expression by Langerhans' cells was significantly increased in koilocytic lesions and in CIN grades I and II, and HLA-DQ expression was significantly increased in all grades of CIN with the increase being most pronounced in CIN I (table 3). Thus although Langerhans' cells are reduced in number compared with normal epithelium, those present seem to be more highly "activated".

The parallel increase in HLA-DR and HLA-DQ expression by Langerhans' cells in CIN is consistent with reports from other groups that HLA-DQ expression correlates in general with HLA-DR.²⁴ HLA-DQ is almost always expressed at a much lower level than HLA-DR,²⁴ so that detection of HLA-DR positive, HLA-DQ negative cells may reflect the limit in sensitivity of the technique used.

Most published work suggests that the expression of HLA-DQ and HLA-DP antigens have the same functional importance as the expression of HLA-DR.²⁵⁻²⁷ Navarette *et al*,^{28,29} however, proposed that HLA-DQ has a function distinct from HLA-DR. They report that while HLA-DR is implicated in the induction of the proliferation of helper/inducer T lymphocytes, HLA-DQ has a predominantly modulatory function entailing the generation of suppressor or cytotoxic T lymphocytes, or both. This may be of relevance to the present study, as an increase in the relative proportion of cytotoxic/suppressor T cells has been reported previously in CIN.³⁰ The increase may be related to the increased expression of HLA-DQ which we found to be particularly noticeable in CIN I, and which may permit a more rapid progression of the neoplastic process.

COMBINED USE OF CONTRACEPTIVE PILL AND MHC CLASS II EXPRESSION

In this part of the study we found that Langerhans' cells were increased in number in the epithelium of the cervical transformation zone of users, compared with non users, and that this increase was significant in patients with CIN I (table 4). This has not been

Fig 3 DA6.231 staining of normal columnar epithelium; adjacent squamous epithelium is negative.

previously reported, although other workers have shown an impairment of non-specific peripheral blood lymphocyte proliferation in users³¹ and an increase in cervical mucus IgG and IgA.³² Perhaps the increased numbers of Langerhans' cells help start and amplify a local antibody response in the cervix. In any case it is interesting to note a difference between users and non-users in view of the continuing controversy over the role of the pill in the aetiology of cervical cancer.^{33 34}

When smokers were compared with non-smokers, no difference in the number of Langerhans' cells or class II expression by Langerhans' cells could be detected (data not shown).

EXPRESSION OF MHC CLASS II ANTIGENS BY COLUMNAR AND SQUAMOUS CERVICAL EPITHELIUM

The expression of class II antigens by columnar epithelium lining endocervical glands was a chance finding as most sections studied contained no columnar epithelium. Expression of HLA-DR by endometrium has been reported to vary with the menstrual cycle³⁵ being highest in the late proliferative phase and thus correlating with oestrogen receptor content. No apparent correlation between intensity of staining and stage of the menstrual cycle was found in the present study, but 11 of 19 subjects in this group were taking the pill and therefore not experiencing normal cycles. It would be useful to examine this in more detail.

In some cases (14%) of CIN III the squamous epithelium exhibited positive staining with DA6.231, although no squamous epithelium from normal cervix or from areas of CIN I or II was positive. Morris *et al* reported HLA-DR expression by squamous epithelial cells in CIN III and, in patches, in koilocytic lesions with no associated CIN.⁵ It is postulated that keratinocytes³⁶ and endometrial epithelial cells³⁵ are induced to express class II antigens by lymphokines secreted by infiltrating lymphocytes and thus acquire the ability to act as antigen presenting cells. Our detection of class II positive epithelial cells in CIN III may be further evidence to support this hypothesis. Functional assays should bring further understanding of the interactions taking place between Langerhans' cells, lymphocytes, and epithelial cells in the cervical transformation zone and of the clinical importance of class II antigen expression at this site.

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