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HLA Expression in Pre-invasive Cervical Neoplasia in Relation to Human Papilloma Virus Infection

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A significant proportion of cervical carcinomas show loss of major histocompatibility complex human leucocyte antigen (HLA) class I expression while upregulating HLA class II expression. These changes may have direct consequences for immune surveillance of the human papilloma virus (HPV) infection which is strongly associated with cervical malignancy. A relationship between changes in HLA expression and HPV infection may be evident in the evolution of premalignant disease. This immunohistological study of 104 colposcopic biopsies establishes that HLA class II expression occurs in a significant proportion of squamous epithelia showing histological evidence of wart virus infection and cervical intraepithelial neoplasia (CIN) I to III. In comparison, alteration of HLA class I expression in cervical premalignant lesions is rare. There is no correlation between the detection of high risk HPV DNA (types 16, 18, 31 and 33) by polymerase chain reaction (PCR) and the MHC class II phenotype of the lesion. This suggests that altered HLA class II expression is neither a consequence nor a prerequisite for HPV infection.

Keywords: HLA, cervical intraepithelial neoplasia, human papilloma virus, MHC class II.
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INTRODUCTION

CERVICAL CANCER and precancer form a disease continuum ranging from cervical intraepithelial neoplasia (CIN) through microinvasion to invasive carcinoma; about 70% of the tumours are squamous and 30% are adeno- and adenosquamous carcinomas [1]. Most tumours are thought to develop from an area of intra-epithelial neoplasia within the transformation zone [2]. This is at the junction of the ectocervical non-keratinising stratified squamous epithelium and the columnar epithelium lining the endocervical canal. At puberty, the increased concentration of ovarian hormones increases the bulk of the cervix leading to eversion of the columnar epithelium. Squamous metaplasia, the gradual replacement of the columnar by squamous epithelium through reserve cell proliferation [3], occurs in response to the relative acidity of the vaginal environment compared to that of the cervical canal. It is in the transformation zone that CIN may arise either by unicellular origin with horizontal spread to replace the normal epithelium [4] or by field transformation [3, 5]. An association between sexual behaviour and cervical cancer has long been observed (e.g. early age at first intercourse [6] and number of sexual partners [7]), and it is recognised that sexually transmitted infections are one of the

major risk factors for cervical carcinoma and the active agents are thought to be specific types of human papilloma virus (HPV) [8]. These high risk HPV types, usually HPV 16 or 18 but also HPV 31, 33 and others are found in over 80% of cervical carcinomas and are commonly associated with high grade CIN [9].

The expression of specific viral proteins through the various stages of the natural history of cervical cancer may evoke immunological consequences of relevance to the progression or not of the disease. Such viral tumour antigens are recognised by specific T cells only in the context of products of the major histocompatibility complex (MHC). The relevance of MHC class I restriction of cytotoxic cells to tumour immunology is highlighted by the observation that the cells transformed by certain oncogenic viruses evade surveillance by downregulation of the expression of MHC class I molecules [10]. This emphasises the need to investigate the tumour expression of MHC products as well as putative tumour antigens such as oncofetal- or virus-related molecules. MHC class II molecules restrict helper T cell responses and are usually expressed by antigen-presenting cells. However, MHC class II antigen expression can occur in carcinomas derived from a class II negative tissue, and this altered expression can be associated with both better and poorer prognosis for the patients [11, 12]. Our previous studies in cervical cancer explored the relationship between the presence of HPV and changes in the MHC class I products [13]. There was no direct correlation between the presence of HPV DNA and altered class I expression. However, the earlier stage cancers which showed class I downregulation had a significantly poorer clinical outcome [14]. We have recently demonstrated that the majority of squamous carcinomas express MHC class II antigens, although normal cervical squamous epithelium is class II negative [15]. There was no apparent correlation between the class II phenotype and the presence of HPV 16 DNA in the specimens. The pathogenesis of cervical cancer is a multifactorial and multistage process, and a relationship between MHC class I and/or class II expression and HPV infection may be evident in the evolution of premalignant disease.

This study was undertaken to examine the pattern of class I and II expression in pre-invasive neoplasia and in cervical epithelia showing evidence of human papilloma virus infection (HPVI).

MATERIALS AND METHODS

Clinical material

Biopsies of clinical material were obtained from patients attending either the Christie Hospital, St. Mary's Hospital for Women and Children or Withington Hospital (University Hospital of South Manchester). Approval from the Ethical Committee of the South Manchester Health Authority and St. Mary's Hospital was obtained.

Biopsies of normal cervix and normal vagina were obtained from patients undergoing total abdominal hysterectomy for benign disease of the uterine corpus, or vaginal hysterectomy for utero-vaginal prolapse, at either St. Mary's Hospital or Withington Hospital. Patients were selected on the basis of no history of cytological abnormality and at least two documented normal cervical smears, the last one being taken within 12 months of the surgery. Cervical biopsies were taken from the fresh hysterectomy specimen, and small vaginal biopsies were taken from the vaginal vault before surgical closure. The biopsies were confirmed as being normal by histological assessment of cryostat haematoxylin and eosin (H&E)-stained sections of the tissue.

Frozen colposcopic biopsies of cervix, and in some cases vagina, were obtained from patients attending a colposcopy clinic at St. Mary's Hospital. Most patients had been referred for colposcopic evaluation of the cervix following abnormal cervical cytology, and had received no previous treatment. A minority of women were being followed up after treatment for CIN, vaginal intraepithelial neoplasia (VAIN) or multiple intraepithelial neoplasia (MIN). If the biopsy was small then the tissue was used only for routine histological assessment. When of sufficient size, biopsies were divided: one portion was fixed in eosin-tinted buffered formalin and subsequently paraffin wax-embedded and routinely processed for diagnostic purposes; the other was snap frozen in liquid nitrogen. Tissues were processed as described previously [15] and every ninth section was analysed histologically for grade of lesion using previously published criteria [16]. One hundred and four colposcopic biopsies were obtained: 85 were cervical biopsies from patients who had received no previous treatment for pre-invasive neoplasia, 10 were cervical biopsies from patients attending the follow-up clinic after receiving laser treatment for pre-invasive cervical disease and 9 biopsies were of vaginal tissue from patients who had either suspected or confirmed VAIN.

Archival wax-embedded specimens from 57 cervical cancer and 6 MIN patients were obtained from St. Mary's Pathology Laboratory. Frozen and paraffin wax-embedded biopsies of cervical carcinomas were obtained from patients referred to the Christie Hospital for radiotherapy treatment of a histologically diagnosed cervical carcinoma as described previously [15]. To detect mucins, periodic acid–Schiff/alcian blue stains with and without diastase, with the alcian blue being used at a pH of 2.4, were also performed [1].

Immunohistochemistry

An indirect immunoperoxidase technique was used for detecting HLA class I and II antigens in frozen tissue sections as described previously [15]. An alkaline phosphatase–anti–alkaline phosphatase (APAAP) procedure was used for detection of HLA class II antigens in routinely processed, formalin-fixed, wax-embedded tissue. Sections were dewaxed by two 5-min immersions in xylene and immersion in 100% alcohol. After air-drying, each section was surrounded with a silicon marker and then incubated in water at 37°C for 5 min. A trypsin digestion procedure, which improves the intensity of the immunostain with CR3/43 antibody, was then carried out for 15–30 min by immersing the slides in 0.05 mol/l Tris–HCl, pH 7.8, containing 0.01% calcium chloride and 0.01% trypsin (1120 $N\alpha$-benzoyl-L-arginine ethyl ester U/mg). Specimens were then incubated sequentially with 10% normal rabbit serum, primary monoclonal antibody (MAb), rabbit anti-mouse immunoglobulin in Tris-buffered saline (TBS) containing 10% normal human serum, mouse monoclonal anti-alkaline phosphatase and soluble

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complexes of alkaline phosphatase. The second and third layer antibody steps were repeated twice which intensifies the final colour reaction when the substrate (2 mg napthol AS-MX phosphate in 200 μl dimethylformamide, made up to 10 ml 0.1 mol/l Tris–HCl, pH 8.2 with 15 μl 1 mol/l levamisole, to inhibit endogenous alkaline phosphatase, plus 10 mg fast red TR salt) was added. Thirteen specimens, omitted from the data presented, did not stain for HLA class II antigens after 15, 20 or 30 min of digestion. The presence of HLA class II expressing leucocytes in the tissue sections acted as internal positive controls as to whether or not the staining procedure had been successful in individual specimens.

The murine MAb used to investigate the expression of MHC class I and II molecules by normal and malignant cervical epithelial cells were as described previously [15]: CR3/43 (1/50) detecting a monomorphic HLA class II antigen; TAL.1B5 (1/50) detecting HLA-DR; DA6.164 (1/10) detecting HLA-DR except HLA-DR7 and some HLA-DQ; B7/21.1 (1/100) detecting HLA-DP; TU22 (1/1000) detecting HLA-DQ and some HLA-DR; W6/32 (1/100) detecting a monomorphic HLA class I antigen (SeraLab, Crawley Down, U.K.); LP34 (1/5) detecting cytokeratins 6 and 18, (SeraLab); and MNF 116 (1/50) detecting cytokeratins 10, 17 and 18 [17]. Negative (no first layer antibody) and positive (LP34 cytokeratin-specific MAb or W6/32 recognising MHC class I molecules) controls were included for each cryostat specimen. In addition, MHC class II expression of the leucocytes infiltrating the stroma served as an internal control in each section. The location of the epithelial tumour cells was assessed by labelling with antibodies recognising cytokeratins expressed by both normal and neoplastic epithelial cells; MAb LP34 for cryostat and MAb MNF 116 for wax sections.

Specimens labelled with anti-class I or class II antibodies were classified as uniformly positive (+, ++, +++), heterogeneous (+/−) or uniformly negative (−). The number of + is proportional to the intensity of staining: +++ strongly expressed; ++ moderately expressed; + weakly expressed. The relative dominance of expression of the class II sub-locus antigenic products was assessed by area and intensity of the labelling in sequential sections.

Detection of HPV DNA

The presence of HPV DNA was assessed in 81 of the snap-frozen cervical biopsies as described previously [18]. This is a two-step polymerase chain reaction (PCR) procedure for the detection of HPV genotypes. A prescreen of samples is performed with a general primer-mediated PCR method to detect a broad spectrum of sequenced and still unsequenced HPV types at the subpicogram level [19]. The HPV-containing scrapes are then subjected to HPV 6, 11, 16, 18, 31 and 33 type specific PCR using anti-contamination primers to identify the sequenced HPV types. The method was applied to crude frozen tissue sections. Inbetween cutting individual cervical biopsy specimens, the cryostat and knife were cleaned and a control liver section cut and collected for PCR. Frozen tissue sections were subjected to a freeze–thaw step followed by heating at 100°C for 5 min in 250 μl distilled water. After cooling on ice and centrifugation for 1 min at 3000 *g*, 40 μl of the sample extracts were used in each PCR assay. The general primer-mediated PCR was performed in 50 μl reaction mixture containing 50 mmol/l KCl, 10 mmol/l Tris Cl (pH 8.3), 3.5 mmol/l $MgCl_2$, 200 mmol/l of each nucleoside triphosphate, 50 pmol of primer GP5 (5′-TTTGTTACTGTGGTAGATAC-3′), 50 pmol GP6 (5′-GAAAAATAAACTGTAAATCA-3′) and 1 unit of thermostable DNA polymerase (Ampli-Taq; Cetus, Emeryville, California, U.S.A.). DNA denaturation at 94°C for 5 min was followed by 40 cycles of amplification performed using a PCR processor (Bio-med, Krefeld, Germany). Each cycle included a denaturation step to 94°C for 1 min, an annealing step at 40°C for 2 min and an elongation step at 72°C for 1.5 min. Ten microlitres of PCR mixtures were analysed by agarose gel electrophoresis and subsequent hybridisation with a mixture of GP5/6-directed PCR products derived from HPV 6, 11, 16, 18, 31 and 33 under low stringency conditions. Type-specific PCR was performed as described for general primer-mediated PCR except that 1.5 mmol/l $MgCl_2$, 25 pmol of each primer and an annealing temperature of 55°C were used [18]. Appropriate PCR-positive (1000 Siha cells) and -negative (standard solution and liver sections) controls were included in each PCR assay. The quality of the samples was first determined by PCR with β-globin-specific primers PCO3/PCO4, as described previously [20]. Only β-globin-positive samples were subjected to HPV PCR.

RESULTS

HLA class I expression in cervical colposcopic biopsies (cryostat sections)

Twelve normal cervix specimens were examined; HLA class I antigens were strongly expressed throughout the full thickness of the squamous epithelium in seven specimens, on the lower two thirds in four specimens and on the lower third only in one specimen. Seventy-two frozen cervical colposcopic biopsies were stained for the presence of HLA class I antigens. The following histological diagnoses were represented: normal metaplasia (5 cases); metaplasia with histological evidence of HPVI (4 cases); normal squamous epithelium (7 cases); squamous epithelium with histological evidence of HPVI (19 cases); CIN I (5 cases); CIN II (10 cases); CIN III (21 cases); invasive carcinoma (1 case). Of the 72 cases, 71 had patterns of HLA class I expression seen in normal cervical epithelium: 51 (71%) had class I staining through all epithelial layers, 17 (24%) had staining of the lower two thirds of the epithelium and three (4%) had staining just of the lower third of the epithelium. Only one sample case of CIN II had full-thickness of loss of class I antigen which correlated with the histological dysplasia. In summary, the loss of class I monomorphic determinants was rarely found in pre-invasive lesions.

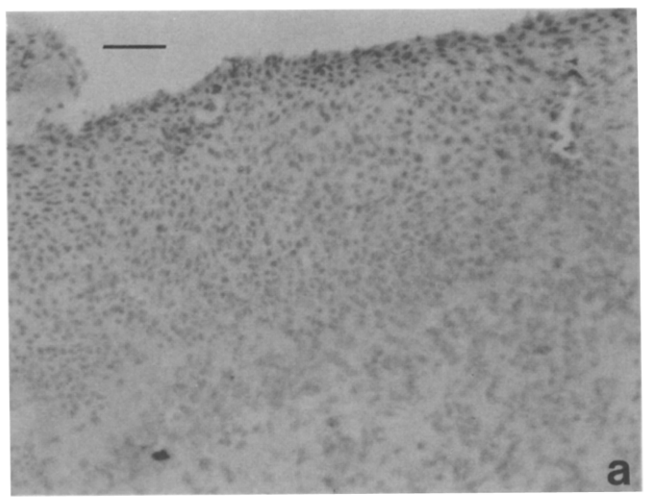
HLA class II expression in cervical colposcopic biopsies (cryostat sections)

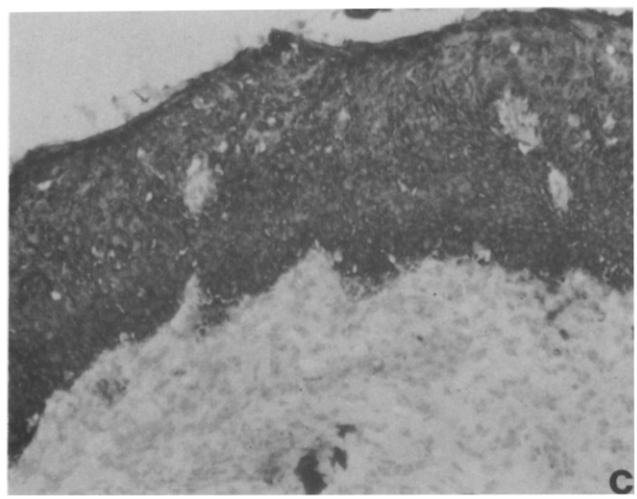
The patterns of HLA class II expression in the different categories of cervical disease are summarised in Table 1. Histologically normal squamous epithelial biopsies from colposcopy patients were always HLA class II-negative as in normal 'control' squamous epithelium. Immature metaplastic squamous epithelium is at different stages of maturation in the transformation zone between columnar and squamous epithelium and 80% are HLA class II-positive. A significant proportion of squamous epithelia with abnormal histology showed MHC class II expression: HPVI (53%), CIN I, II or III (67, 58 and 93%, respectively). There were no significant differences in the proportions of specimens showing HLA class II expression between CIN I, II or III lesions with or without histological evidence of HPV infection (Fisher's exact test $P > 0.05$). An example of class II expression in cryostat section of a CIN III lesion is shown in Fig. 1.

HLA class II expression in cervical lesions was frequently

Table 1. HLA class II expression in pre-invasive cervical disease

Histological diagnosis (*n*)	Mean age (years)	HLA class II expression (immunohistochemistry with MAb CR3/43) Negative	Positive: Heterogeneous	Positive: Homogeneous	Positive: Total positive	Statistics (Fisher's exact test)
Normal squamous (*n*=8)	31.9	8 (100%)	0	0	0	Significant vs. all other groups. $P = < 0.03$
Warty squamous epithelium (*n* = 19)	28.4	9 (47.4%)	6 (31.6%)	4 (21%)	10 (52.6%)	Significant vs. normal epithelium $P = 0.011$
Immature metaplasia (*n* = 5)	25.4	1 (20%)	3 (60%)	1 (20%)	4 (80%)	Significant vs. normal epithelium. $P = 0.007$
Warty immature metaplasia (*n* = 5)	26.0	2 (40%)	1 (10%)	2 (40%)	3 (60%)	Significant vs. normal epithelium $P = 0.015$
CIN I (*n* = 6)	27.8	2 (33.3%)	1 (16.7%)	3 (50%)	4 (66.7%)	Significant vs. normal epithelium $P = 0.016$
CIN II (*n* = 12)	24.0	5 (41.7%)	4 (33.3%)	3 (25%)	7 (58.3%)	Significant vs. normal epithelium $P = 0.015$
CIN III (*n* = 28)	27.8	2 (7.2%)	12 (42.9%)	14 (50%)	26 (92.9%)	Significant vs. normal epithelium $P = < 0.0001$





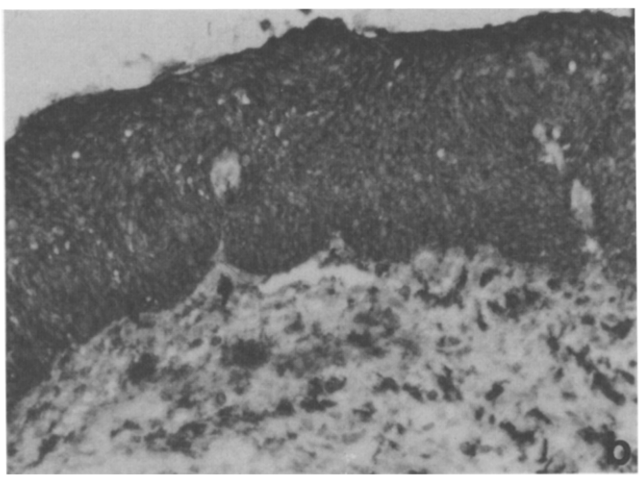


Fig. 1. HLA class II expression in CIN III lesion detected by immunohistochemistry in cryostat sections: (a) control, no primary antibody; (b) CR3/43 detecting HLA class II monomorphic determinants and (c) LP34 detecting epithelial cytokeratins. Bar = 100 μm.

heterogeneous, as has been described previously for squamous carcinomas of the cervix [15]. Class II-positive lesions of this type usually, but not invariably, had a staining pattern in which regions of full thickness epithelium would stain positively adjacent to histologically identical class II-negative areas. Both cytoplasmic and membranous staining was seen. In squamous epithelium that had histological evidence of HPVI, the heterogenous pattern of staining for class II antigen tended to be one in which the basal region was positive and the upper epithelial cell layers were negative. Variations in the staining included: basal staining that could be patchy or membranous staining noticeably stronger than the cytoplasmic staining of the cells. In four specimens, class II antigen expression was detected by CR3/43 reactivity, but staining of adjacent tissue sections for sub-locus expression was negative. This could either be due to a greater sensitivity of MAb CR3/43 in this assay than the MAb used to define sub-locus expression, or the patient being homozygous for HLA-DR7, an antigen that MAb DA6.164 (used to define HLA-DR expression) does not detect.

Eight class II positive lesions exhibited similar staining patterns with both MAb TAL.1B5 and CR3/43 recognising determinants of the α and β chains of HLA class II molecules, respectively. The presence or absence of class II antigens in a lesion showed no correlation with the phase of the menstrual cycle during which the biopsy was performed (data not shown).

Expression of HLA-DR, -DP and -DQ was examined in 44 lesions classified as class II-positive as defined by staining with MAb CR3/43. When divided into histological subgroups, the number of cases in each group are comparatively small (warty, 7; metaplasia, 7; CIN I, 4; CIN II, 6; CIN III, 20). But there were no significant differences in the HLA class II locus expression between groups. In each histological category, HLA-DR was most commonly expressed and HLA-DQ least commonly expressed. In the CIN III group, HLA-DR is expressed

Table 2. HLA class II expression in paraffin-processed archival cervical epithelial lesions

Epithelial category (n)	HLA class II expression			
		Positive		
	Negative	Heterogeneous	Homogeneous	Total positive
Normal squamous (n = 28)	28 (100%)	0	0	0
Normal columnar (n = 33)	0	0	0	33 (100%)
Normal metaplasia* (n = 11)	2 (18.2%)	2 (18.2%)	7 (63.6%)	9 (81.8%)
Warty metaplastia (n = 2)	1 (50%)	0	1 (50%)	1 (50%)
Warty squamous (n = 13)	11 (84.6%)	1 (7.7%)	1 (7.7%)	2 (15.4%)
CIN I (n = 2)	2 (100%)	0	0	0
Warty CIN I (n = 6)	3 (50%)	0	3 (50%)	3 (50%)
All CIN I (n = 8)	5 (62.5%)	0	3 (37.5%)	3 (37.5%)
CIN II (n = 2)	0	2 (100%)	0	2 (100%)
Warty CIN II (n = 5)	1 (20%)	1 (20%)	3 (60%)	4 (80%)
All CIN II (n = 7)	1 (14.2%)	3 (42.9%)	3 (42.9%)	6 (85.8%)
CIN III (n = 7)	2 (28.6%)	2 (28.6%)	3 (42.8%)	5 (71.45)
Warty CIN III (n = 5)	1 (20%)	1 (20%)	3 (60%)	4 (80%)
All CIN III (n = 12)	3 (25%)	3 (25%)	6 (50%)	9 (75%)
Microinvasive SCC (n = 4)	2 (50%)	1 (25%)	1 (25%)	2 (50%)
Adenocarcinoma *in situ* (n = 6)	0	0	6 (100%)	6 (100%)
Reserve cell hyperplasia (n = 3)	0	0	3 (100%)	3 (100%)
Atrophic epithelium (n = 3)	3 (100%)	0	0	0
Microglandular hyperplasia (n = 2)	0	0	2 (100%)	2 (100%)

*Immature normal metaplastic epithelium. SCC, squamous cell carcinoma.

significantly more frequently and a semi-quantitative ranking order indicated that in the majority of cases, class II expression was predominantly due to expression of HLA-DR.

HLA class II expression in formalin-fixed, wax-embedded cervical lesions

Examination of paraffin-processed, wax-embedded archival specimens enabled a wider range of both neoplastic and non-neoplastic epithelial lesions to be studied and their HLA class II phenotypes are summarised in Table 2. There were no statistically significant differences in the proportions of HLA class II-positive specimens of the different histological categories analysed in cryostat versus wax sections including warty epithelium (squamous epithelium with histological evidence of HPVI): 52.6 vs. 15.4%, respectively ($P > 0.05$, Fisher's exact test). In the study of archival specimens in CIN I, CIN II and CIN III categories, there were significant differences in HLA class II expression compared to normal squamous epithelium ($P < 0.01$, Fisher's exact test). No differences were seen in the histologically defined warty subgroups in these lesions ($P = 0.09$, Fisher's exact test). Six cases of adenocarcinoma *in situ* were all strongly class II-positive and of the four cases of micro-invasive squamous cell carcinoma (SCC) examined, two cases were class II-positive and two were class II-negative. Three cases of reserve cell hyperplasia were strongly class II-positive.

In some of the specimens, the demarcation between class II expression of the epithelial lesion coincided with an area of histologically normal squamous epithelium. In several specimens the inflammatory stromal infiltration was sufficiently heavy to merit a diagnosis of 'chronic active cervicitis', and in four specimens a diagnosis of follicular cervicitis (suggestive of a chlamydial infection) was made. There was no correlation between the class II phenotype of the CIN or HPVI epithelium and the adjacent histologically normal areas in any of these cases. These observations are consistent with constitutive upregulation of HLA class II expression in the cervical lesions and not with a response to lymphocyte infiltration *per se*.

HPV detection and HLA class II expression

The data in Table 3 show that there was no relationship between the detection of high-risk HPV DNA and the pattern of HLA class II expression in the lesions. Figure 2 shows that the presence of HPV 16, 18, 31, 33 DNA was associated only with CIN and the proportion of positives increased significantly from CIN I to III ($P = 0.008$).

HLA class II expression in VAIN and MIN

Five frozen biopsies of histologically normal vaginal vault tissue were all HLA class II-negative using MAb CR3/43. Nine samples of VAIN were analysed: five of the epithelia were class II-negative and four cases—two with evidence of HPVI, one case of VAIN III with focal invasion and one case of immature epithelium—were found to express HLA class II molecules. HLA-DR was the predominant antigen in these cases.

Paraffin-processed biopsies from 6 patients suffering from MIN were examined for HLA class II expression. It can be seen from the data in Table 4 that both VAIN, vulval intraepithelial neoplasia (VIN) and peri-anal intraepithelial neoplasia (PAIN) lesions may or may not express class II antigens. There was no consistency of class II expression from biopsies of epithelia removed at different sites from the same patient. For example, case no. M1 had expression of class II antigens in CIN III and VIN III lesions but other biopsies of CIN II and VAIN I–III were class II-negative.

Table 3. HPV and HLA class II expression in cervical lesion biopsies

HPV detection (n)	HLA class II phenotype: Negative	Positive: Hetero-geneous	Positive: Homo-geneous	Positive: Total positives
Negative ($n=10$)	2 (20%)	1 (10%)	7 (70%)	8 (80%)
HPV X, 6, 11 ($n=19$)	9 (47%)	6 (32%)	4 (21%)	10 53%)
HPV 16,18, 31,33 ($n=33$)	11 (33%)	11 (33%)	11 (33%)	22 (67%)
Total ($n=63$)	22	18	22	40
HPVI ($n=10$)	4	3	3	6
CIN I ($n=10$)	5	2	3	5
CIN II ($n=13$)	7	2	4	6
CIN III ($n=29$)	6	11	12	23

There was no correlation between the HLA class II phenotype of the cervical lesion biopsy and the detection of HPV DNA of either low or high risk types (Fisher's exact test $P > 0.05$). In normal cervix specimens ($n=18$), HPV X was detected in four specimens. HPVI had four HPV X and one HPV 6; CINI had seven HPV X, one HPV 6 and three HPV 16; CIN II had three HPV X, one HPV 6, two HPV 16, one HPV 18, two HPV 31 and two HPV 33; CIN III had three HPV X, eighteen HPV 16 and five HPV 33.

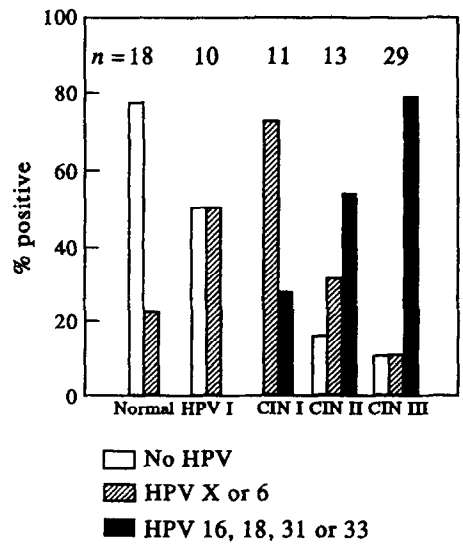


Fig. 2. HPV prevalence as detected by PCR in cervical biopsies. HPV DNA was detected and typed by a combined general primer-mediated/type-specific PCR strategy. Type-specific PCR analysis was for HPV types 6, 11, 16, 18, 31 and 33. Samples that were HPV-positive with general primers but HPV-negative in the type-specific PCR assays were tentatively classified as containing HPV X, indicating all HPV types that differ from HPV 6, 11, 16, 18, 31 and 33, including possible unidentified (novel) HPV. n = number of specimens tested.

DISCUSSION

In contrast to squamous carcinomas of the cervix [13], the incidence of HLA class I loss in premalignant lesions is rare. The loss of HLA class I expression may be a relatively late event in the natural history of cervical cancer but a more interesting

Table 4. HLA class II expression in multiple intraepithelial neoplasias paraffin processed archival specimens

Case no.	Biopsy (year)	Class II phenotype of epithelial lesions
M1	1989	Warty epithelium − CIN II − CIN III +/−
	1989	Warty epithelium − CIN II +
	1989	VAIN I/II /III −
	1989	VIN III +/−
	1989	PAIN +/−
M2	1980	VAIN III +/−
	1983	VIN III +
	1986	VAIN III +
M3	1983	VIN III−
	1985	Hyperplastic vulval dystrophy −
M4	1986	VIN II/III −
M5	1983	VIN III +/− Invasive carcinoma -
	1985	VIN II/III +/−
M6	1975	CIN II −
	1978	VIN III −
	1985	VIN III −

possibility is that the changes will only be evident in premalignant lesions which will progress to invasive disease. Analysis of HLA class II expression in frozen colposcopic biopsies demonstrates that 53% of squamous epithelium with histological evidence of HPVI, 67% of CIN I cases, 58% of CIN II cases and 93% of CIN III lesions have class II expression in all or a proportion of the affected epithelium. None of these proportions are statistically different from the 75% of cervical carcinomas that are class II-positive [15]. HLA class II expression is significantly different in CIN and HPVI compared to the normal squamous epithelium which is class II-negative. Investigation of individual HLA class II loci expression revealed that, as in the invasive lesions [15], HLA-DR expression was the most common, followed by HLA-DP and with HLA-DQ the least frequently expressed. The analysis of archival specimens of cervical premalignant lesions confirmed the results for HLA-DR expression from cryostat sections.

In contrast to normal vaginal epithelium, VAIN and HPVI lesions may express HLA class II antigens. Some VIN and PAIN lesions are class II-positive but within an individual patient there is no overall pattern of class II expression common to all the epithelial lesions. It has been postulated that a common infectious agent or a genetic susceptibility to intraepithelial neoplasias exists in MIN patients, but no inference on the biological role of class II epithelial expression can be drawn from this study.

Reserve cells become apparent at the first stage of squamous metaplasia and the three cases of hyperplasia studied were class II-positive, as were 80% of cases of immature squamous metaplasia. It is tempting to speculate that class II molecules are downregulated during the maturation process to mature squamous epithelium in the transformation zone. Combining all the data for the cervical lesions studied in this report and previously [15], the proportions of specimens showing HLA class II expression are shown in Fig. 3. The precise origin of the reserve cells remains unknown but it is possible that they represent the target cell type for origin of premalignant lesions of the cervix. Other influences may independently regulate HLA class II expression but this could be fixed, possibly by HPV infection, and account for the similar proportions of class II-positive adeno-, adenosquamous and squamous carcinomas of the cervix with respect to class II phenotype [15]. Some previous studies of small numbers of HPVI and CIN lesions have shown expression of HLA class II antigens [21, 22, 24], whereas others have not [23]. The data presented here clearly establish that a significant proportion of pre-invasive cervical lesions express HLA class II antigens.

High-risk HPV [16, 18, 31, 33] types were detected in CIN I (27%), CIN II (54%) and CIN III (79%) and not in normal metaplastic epithelia with or without warty changes. HPV 16 was the most frequent type detected in the CIN specimens (43%). The distribution of infection with the low-risk or high-risk HPV in the cervical specimens studied is similar to that of another study using cervical smears, although incident rates are slightly lower [25]. The different detection rates may reflect population or sampling differences between the U.K. and the Netherlands. There was no correlation between the groups of cervical lesions exhibiting either no HPV, low-risk or high-risk HPV infections and the HLA class II phenotypes. This suggests that the latter is not a consequence or a requirement of HPV infection in the cervix.

The biological and clinical implications of MHC class II expression on epithelium with HPVI or CIN are unknown. The sharp demarcation of class II expression at the interface of CIN III/normal squamous epithelium and the lack of correspondence of class II expression with heavy sub-epithelial leucocyte infiltrates in normal epithelium, CIN or HPVI is inconsistent with local cytokine production being the sole cause of epithelial cell class II expression. HPV infection might impair the normal afferent arm of the immune system, since in both HPVI [21, 22] and CIN [28, 29], reductions in epithelian Langerhans' cells have been reported. The constitutive expression of HLA class II by squamous epithelia with dysplasia may allow antigen presentation to infiltrating lymphocytes within the epithelium with either positive or negative consequences for progression of the cervical lesion. HLA class II restricted T-helper/inducer lymphocytes might either facilitate a specific antibody response

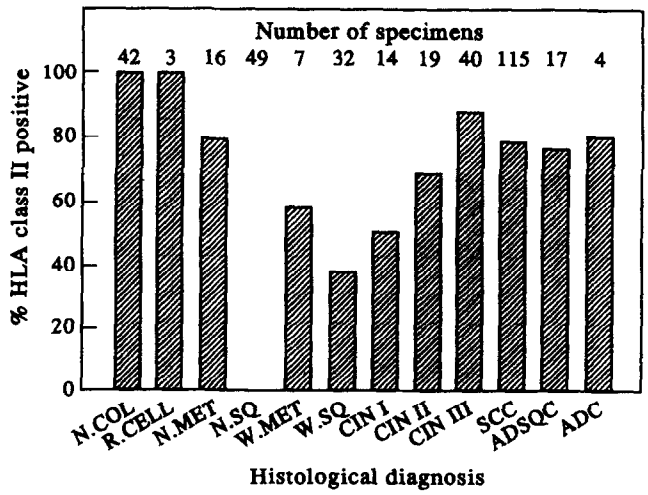


Fig. 3. HLA class II expression in epithelia of the cervix in normal and abnormal physiological states: normal columnar epithelium (N.COL), reserve cell hyperplasia (R.CELL), normal metaplasia (N.MET), normal squamous epithelium (N.SQ), warty (HPVI) metaplasia (W.MET), warty (HPVI) squamous epithelium (W.SQ), CIN I, II, III, squamous cell carcinoma (SCC), adenosquamous carcinoma (ADSQC) and adenocarcinoma (ADC). The data were pooled from the cryostat and wax section analysis as well as from [15].

or a state of anergy in the T cell clones that recognise a relevant antigen [26] or immunosuppression by recruitment of specific T-supressor, possibly HLA-DQ-restricted $CD8^+$ cells [27].

A carefully controlled prospective follow-up study of HPVI lesions of the cervix over a period of 6 to 9 months, in which sequential cervicographical records are made of lesions initially evaluated by colposcopy and histology, may help evaluate whether class II expression by an infected area of epithelium is linked to regression of the lesion. It is interesting that there is a linkage of regression and conversion of rabbit viral papillomas with MHC class II genes [30].

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