Immunoreactive α interferon in cervical flat koilocytic lesions and intraepithelial neoplasia

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Abstract

Immunocytochemical staining for α -interferon was carried out on cervical biopsy specimens showing non-condylomatous koilocytic atypia (n = 12) and cervical intraepithelial neoplasia (n = 18), both of which are associated with human papilloma virus (HPV) infection. Normal cervical tissue obtained from hysterectomy specimens was also assessed. Koilocytes were not immunoreactive for α interferon and keratinocyte staining was observed in only four cases of intraepithelial neoplasia. HPV infection alone does not therefore seem to induce the production of α interferon in cervical squamous epithelium. There was variable but, in some cases, prominent staining of cells in the stromal inflammatory infiltrate as well as intraepithelial cells which had morphological and immunocytochemical characteristics of Langerhans' cells. Alpha interferon immunoreactivity in Langerhans' cells is in keeping with derivation from the mononuclear phagocyte system and may be important in the host response to HPV infection.

The interferons are a family of proteins which have the ability to protect cells from viral infection.¹ Three major classes of interferon are now recognised and designated α , β , and γ . Interferon is an important factor in the host response to viral infection in man, and interferon activity in vivo probably involves a direct anti-viral effect mediated via cell surface receptors and effects secondary to modulation of lymphocyte and macrophage function.^{2,3}

Interferon activity in vitro has been extensively investigated but its production or localisation in human tissue in vivo has received less attention. Recently, however, it has been shown that α -interferon immunoreactivity is present in most normal human tissues, particularly in cells of the mononuclear phagocyte system.⁴ It might also be expected that interferon would be present or increased in virally infected tissue.

Epidemiological studies have implicated a sexually transmitted infectious agent in the pathogenesis of carcinoma of the cervix,⁵ and there has been considerable interest in the strong association between human papilloma virus (HPV) infection and tumours of the anogenital tract. The association of cervical condyloma with cervical intraepithelial neoplasia (CIN) is well documented,⁶ and papilloma virus structural antigens have been shown in a proportion of these lesions using immunocytochemical methods.^{7,8} More recently DNA hybridisation techniques have shown various subtypes of HPV in most biopsy specimens from patients with CIN or severe dysplasia.^{9,10} Alpha interferon has recently been used in the treatment of anogenital lesions, associated with HPV,¹¹ but host interferon production in response to viral infection has not been documented. We report here the distribution of α interferon immunoreactivity in normal cervix, cervical flat koilocytic lesions, and in CIN.

Colposcopically directed diagnostic punch biopsy specimens of cervix were selected from patients referred to a colposcopy clinic because of an abnormal cervical smear. The biopsy specimens were fixed in Bouin's solution and processed to paraffin wax. Sections 4 μ m thick from three tissue levels were stained with haematoxylin and eosin. Cases selected showed histological changes associated with HPV infection (n = 12), CIN I (n = 6), CIN II (n = 6) and CIN III (n = 6). All cases of HPV showed non-condylomatous koilocytic atypia (flat koilocytic lesions¹²), and some biopsy specimens also showed individual cell keratinisation and multinucleation. Intraepithelial neoplasia was diagnosed and graded according to the criteria of Buckley, Butler, and Fox.¹³

Methods

Control cases (n = 6) consisted of cervical blocks taken from hysterectomy specimens from patients with no history of cervical disease. The blocks were fixed and processed as above.

Sections were cut from paraffin wax embedded blocks and mounted on slides coated with poly-L-lysine. Sections were stained by an indirect immunoperoxidase technique using diaminobenzidine substrate. The primary antibody was a polyclonal antiserum (H51, gift from Dr A Meager) raised in sheep to human lymphoblastoid interferon (Hu A-IFN ly Namalwa, Wellferon, Wellcome Research Laboratories, Beckenham, Kent).¹⁴ In a viral culture inhibition assay this antibody neutralised all α interferon preparations but also showed very weak neutralisation of human β interferon: γ interferon was not neutralised.¹⁵ The antiserum reacted with α interferon (Wellferon) but not with recombinant β interferon (Triton Biosciences) in immunoblot assay.

The antiserum was preabsorbed with liver

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powder and used at a dilution of 1/100 as previously described.¹⁶

Controls included replacement of the primary antibody by normal sheep serum (diluted 1/100) and antibody preabsorbed with Wellferon or recombinant β interferon.

Serial 4 μ m sections were also cut from selected cases and stained using antibodies to class II major histocompatibility complex (TAL-IB 5), S100 protein (Dako), and with a monoclonal antibody to macrophages (KP 1, gift from DY Mason, Oxford).¹⁷

Results

The staining with the H51 antiserum described below was considered specific and "positive" where immunoreactivity was abolished by prior incubation of antiserum with Wellferon, and where no staining was observed following substitution of the primary antiserum by normal sheep serum.

NORMAL CERVIX

Weak α interferon immunoreactivity was observed in occasional rounded or spindle shaped cells within subepithelial tissue, and uncommonly in dendritic cells in squamous epithelium. Keratinocytes were uniformly negative and endocervical columnar cells also showed no immunoreactivity. Occasional positively stained subcolumnar cells were seen in endocervix and in areas of immature squamous metaplasia.

FLAT KOILOCYTIC LESIONS

Keratinocytes, including those in which koilocytic change was present, showed no evidence of staining. The most prominent staining in all sections was seen in non-keratinocytic cells in squamous epithelium, although in some cases these were only faintly positive. These cells had a rounded, spindle shaped, or dendritic morphology and lay between keratinocytes, particularly in basal to

Figure 1 Flat koilocytic lesion. Alpha interferon immunoreactive cells are present in basal and intermediate zones of squamous epithelium and in subepithelial stroma. Keratinocytes are not stained.

Figure 2 Higher magnification of figure 1. At this magnification the dendritic cytoplasmic processes of α interferon immunoreactive cells are evident between non-stained koilocytes.

intermediate zones of the epithelium (figure 1). The dendritic cytoplasmic processes were frequently prominent and extended around adjacent negatively stained keratinocytes (figure 2). Staining of consecutive serial sections showed that some of these dendritic cells also expressed class II MHC (figure 3), but staining for S100 protein was negative in all cases.

As in control cases α interferon immunoreactivity was also observed in cells in subepithelial tissue, particularly in biopsy specimens with a pronounced stromal inflammatory infiltrate (figure 4). Many of these cells had morphological features of macrophages and some of these were stained by the KP1 antibody. Occasional immunoreactive stromal cells were spindle shaped or dendritic, similar to the cells in squamous epithelium.

Endocervical cells did not show α interferon immunoreactivity, but staining of occasional small subcolumnar cells was present in most biopsy specimens, particularly in areas with a periglandular chronic inflammatory infiltrate (figure 5).

CERVICAL INTRAEPITHELIAL NEOPLASIA

Alpha interferon immunoreactivity was seen in neoplastic squamous epithelium in four of 18 cases. Staining was of weak to moderate degree and observed in all layers of the epithelium (figure 6). As in koilocytic lesions, however, the most prominent staining in squamous epithelium was seen in non-keratinocytic cells many of which exhibited a dendritic morphology (figure 7). Positively stained dendritic cells seemed to be more numerous in CIN than in cervical koilocytic lesions on subjective as-

Figure 3 Flat koilocytic lesion. Consecutive serial sections showing coexpression of α interferon (a) and class II MHC (b) in non-keratinocytic cells in squamous epithelium (arrows).

assessment, although no attempt was made to quantify these cell populations. The intensity of α interferon immunoreactivity in dendritic cells also tended to increase with progression of CIN grades.

Many of the biopsy specimens showed moderate cervicitis, usually with a predominant chronic inflammatory cell component. Alpha interferon immunoreactivity was again observed in spindle shaped, dendritic, and macrophage-like cells in the inflammatory infiltrate, and dendritic cells were often numerous and most intensely stained in epithelium overlying inflammatory foci.

Discussion

Although the association between HPV and cervical neoplasia is well established, relatively little is known about the nature and importance of the host response to HPV infection in the genital tract. It seems likely that the persistence or progression of cervical lesions induced by HPV will depend partly on the development of

an effective immunological or inflammatory reaction to the virus, and this is indirectly supported by evidence that immunosuppressed patients have an increased incidence of cervical neoplasia.¹⁸ As interferon is considered to be an important host antiviral factor, we examined the distribution of immunoreactive α interferon in cervical disease associated with HPV.

HPV is thought to infect the basal cells of squamous epithelium, and as maturation and differentiation occur production of viral structural proteins takes place and morphological changes of viral infection are identified.¹⁹ Immunoreactive α interferon was not, however, shown in keratinocytes in cervical koilocytic lesions in this study and was detected in only four of 18 cases of CIN. This was an unexpected finding because it is generally believed that all cells are capable of producing α interferon, and, furthermore, cultured skin keratinocytes contain α interferon activity²⁰ and produce interferon in response to herpes virus infection in vitro.²¹ Cervical squamous epithelium may respond differently to epidermis and virally infected cervical epithelium may produce other classes of interferon or subtypes of α interferon not detected by the H51 antiserum used in this study. Alternatively, it may be that HPV is not a potent inducer of α interferon; this does not seem to have been examined, possibly because HPV cannot be propagated in tissue culture and is therefore resistant to many routine investigative techniques. If this is the case then one of the principal host survival antiviral responses might be diminished and this could partly explain the characteristic ability of HPV to persist in human tissue.

Figure 4 Flat koilocytic lesion with squamous metaplasia extending into an endocervical crypt. Alpha interferon immunoreactive cells are scattered through stromal inflammatory infiltrate and are present in squamous epithelium.

Figure 5 Endocervical gland with pronounced stromal inflammatory cell infiltrate. Alpha interferon immunoreactive cells with variable morphology are present in the stroma and occasionally in a subcolumnar situation (arrows). Endocervical epithelium is not stained.

Figure 6 CIN III with extension into endocervical crypt. There is weak keratinocytic cytoplasmic staining with more obvious interferon immunoreactive dendritic cells (arrow). Endocervical epithelium is, by contrast, not stained.

The clinical importance of keratinocyte staining in a few cases of CIN is uncertain. As HPV infection alone does not readily seem to induce production of α interferon, it may be that this is a property of the neoplastic epithelium in these cases. It has been shown that all classes of interferon profoundly and reversibly inhibit epidermal keratinocyte proliferation in vitro,^{22,23} and it has been suggested that α interferon or an interferon related protein may act as a chalone and be a regulator of epidermal growth in vivo.²¹ If this is so then localisation of interferon in neoplastic squamous epithelium

could be related to the abnormal cell proliferation which characterises CIN. Most cases of intraepithelial neoplasia, including CIN III, however, showed no keratinocyte α interferon immunoreactivity.

The aim of this study was to examine α interferon immunoreactivity in lesions of cervical squamous epithelium which were thought to be associated with HPV. Unexpectedly, however, staining was most evident in non-keratinocytic cells which comprised a morphologically heterogeneous group. Many of the α interferon positive cells in subepithelial connective tissue had morphological and immunohistochemical features of macrophages. This is in keeping with the demonstration of α interferon immunoreactivity in cells of the mononuclear phagocytic system in most human tissues.⁴ It has been proposed that basal interferon production by these cells may have a physiological role in the continuous response to infectious agents.²⁴

In squamous epithelium positively stained cells commonly exhibited prominent dendritic cytoplasmic processes which extended between adjacent keratinocytes. Coexpression of class II MHC was also present in some of these dendritic cells and these features are consistent with identification as Langerhans' cells.²⁵ Langerhans' cells have previously been shown to be present in cervical epithelium as well as epidermis and are considered to have a similar antigen presenting role at this site.²⁶ We were unable to show the presence of S100 protein, another commonly used immunocytochemical marker for Langerhans' cells in the α interferon positive cells in this study, but this could be

Figure 7 CIN III. Strongly α interferon immunoreactive cells with rounded or dendritic morphology are present but neoplastic keratinocytes are not stained.

explained by the almost complete depletion of S100 positive Langerhans' cells which has previously been described in both HPV infection and intraepithelial neoplasia in the cervix.²⁷ The demonstration of immunoreactive α interferon in Langerhans' cells is not surprising as these cells are considered to be specialised derivatives of the mononuclear phagocyte system.²⁸

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