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# The relationship between epithelial Ia expression and the inflammatory cell infiltrate during experimental oral carcinogenesis

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**Summary.** The development of oral epithelial expression of Ia antigens and its relationship to the presence of IL-2r$^+$ (CD25$^+$) cells was investigated in rats treated with the water soluble carcinogen 4-nitroquinoline-N-oxide (4NQO). Acetone fixed frozen sections of the palate and tongue were stained using an indirect immunoperoxidase technique and monoclonal antibodies to rat Ia (I-A & I-E) and IL-2 receptor. After 4 weeks 4NQO treatment all rats expressed oral epithelial Ia but thereafter (2–9 months) expression was present in only 20–40% of animals. Epithelial expression of Ia by histologically normal, dysplastic and neoplastic epithelium was always associated with the presence of an underlying inflammatory cell infiltrate containing CD25$^+$ cells. Overall there were significantly more CD25$^+$ cells in tissue specimens containing Ia$^+$ epithelium compared with Ia$^-$ epithelium. Furthermore, during the first 4 weeks of carcinogen treatment, a significant positive correlation was found between the CD25$^+$ cell density and occurrence of focal epithelial Ia expression. These results, together with analysis of the T cell, NK cell, macrophage and B cell content of the infiltrates induced by 4NQO, suggest that the CD25$^+$ cells represent activated T cells. Thus, our results in this experimental model are consistent with the idea that epithelial expression of Ia is the result of production of IFN-$\gamma$ by locally activated T cells.

**Key words:** Carcinogenesis – Epithelium – Ia antigens – Il-2 receptor – Inflammatory cells

## Introduction

The induction of T lymphocyte proliferation requires the sequential action of soluble factors produced in response to antigenic stimulation. The combination of antigen presentation and interleukin 1 production by macrophages and/or Langerhans cell induces T lymphocytes to produce interleukin 2 (Il-2) (Larsson et al. 1980; Smith et al. 1980; Durum and Gershon 1982). The latter then stimulates cell division in T lymphocytes bearing Il-2 receptors (Il-2r) and the production of lymphokines including interferon-$\gamma$ (IFN-$\gamma$) (Farar et al. 1981; Pearlstein et al. 1983; Kasahara et al. 1983).

Major histocompatability complex (MHC) class II (Ia) antigens are expressed either constitutively on some cells essentially of bone marrow origin (Acolla et al. 1984) or variably following activation by external stimuli (Halloran et al. 1986). In tumours of epithelial origin, a variable proportion of lesions express Ia (Thompson et al. 1982; Daar and Fabre 1983; Carrel et al. 1986) the stimulus for which is unknown. Although MHC class II expression on normal and malignant cells in vitro can be induced by IFN-$\gamma$ (Basham et al. 1984; Schwartz et al. 1985; Volc-Platzer et al. 1985) this is not an invariable property of all cells in a normal population or all tumour cell lines (Pfizenmaier et al. 1985; Moore et al. 1986). If IFN-$\gamma$ is directly involved in the stimulation of epithelial Ia expression in vivo, the distribution of Ia positive epithelial cells in situ should correspond to the location of activated T cells and macrophages.

In order to investigate epithelial expression of Ia during tumour development and its relationship to the underlying inflammatory cell infiltrate we have used the 4-nitroquinoline N-oxide (4NQO) rat carcinogenesis model which has been shown to induce oral squamous cell carcinomas that are comparable to the human situation (Wallenius and Lekholm 1973). In this model, there is progressive epithelial dysplasia, development of an inflamma-

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tory cell infiltrate and epithelial expression of Ia, prior to tumour development in all animals after approximately 9 months 4NQO treatment (Prime et al. 1986; Matthews et al. 1986). This paper describes an immunocytochemical study demonstrating a close correlation between the development of epithelial Ia reactivity and local presence of Il-2r$^+$ (CD25$^+$) inflammatory cells.

## Materials and methods

*Animals and tissues.* One hundred and fifty two male Sprague Dawley white rats (OLAC Ltd, UK) aged 6–8 weeks were housed in polyethylene cages, a maximum of four rats per cage, and were fed and watered ad libitum. The animals were randomly divided into four groups:

1) To investigate the pattern of epithelial Ia reactivity in tissues exposed to prolonged carcinogen application prior to the development of palatal and lingual carcinomas, fifty four rats in Group I were painted with 0.5% (W/v) 4NQO (Sigma) in propylene glycol which was applied three times weekly to the palates of unanaesthetised rats. Six animals were killed at monthly intervals up to a maximum of 9 months. Although some rats in Group I did develop squamous cell carcinomas during the later stages of carcinogenesis (28 weeks, 3 of 6 rats; 32 weeks, 4 of 5 weeks; 36 weeks, all of 5 rats), there was no evidence microscopically of carcinoma in the tissue specimens examined for Ia reactivity.

2) To examine the possible correlation between the pattern of epithelial Ia reactivity and the nature of the underlying inflammatory cell infiltrate in lingual carcinomas, twelve rats in Group II were treated similarly with 4NQO until overt tumour development at approximately 9–10 months (Wallenius and Lekholm 1973; Prime et al. 1986).

3) To investigate whether the inflammatory cells that were associated with Ia$^+$ malignant epithelium in Group II were also dominant during the initial development Ia reactivity, sixty rats in Group III were treated with 4NQO and five were killed at 2–3 day intervals up to a maximum of 28 days. The 0–28 day time period was chosen because the results from Group I animals demonstrated that all rats showed epithelial expression of Ia at 28 days (see Results section I).

4) Control (Group IV) included eighteen animals painted with solvent alone which were killed in pairs at a) monthly intervals up to 9 months (Group I controls), or b) 7 day intervals up to 28 days (Group III controls) and eight untreated rats which were sacrificed at the conclusion of the experiments.

Four mm thick transverse (mediolateral) tissue blocks were prepared from the lingual tissue immediately posterior to the prominent intermolar tubercle on the dorsum of the tongue and from the palatal mucosa between the last molar tooth. These non-invasive tissues (Groups I, III and IV) and the lingual tumours (Group II) were snap-frozen in thawing isopentane and stored in liquid nitrogen.

*Immunocytochemical staining.* Immunocytochemical staining was performed using a triple layer, two conjugate immunoperoxidase method. Briefly, diluted monoclonal antibodies (Table 1) were applied to acetone-fixed, air dried cryostat sections (5 μm thick) and incubated at room temperature for 60 min. After washing, sections were overlayed with peroxidase-conjugated rabbit anti-mouse immunoglobulin (Dako AS, Denmark; 1:50 dilution in buffer containing 25% normal rat serum) for 30 min at room temperature. After further washing, sections were overlayed with peroxidase conjugated swine anti-rabbit immunoglobulin (Dako AS, Denmark; 1:50 dilution in buffer containing 25% normal rat serum) for 30 min at room temperature. Unbound conjugate was removed by washing and bound peroxidase visualised using diaminobenzidine (DAB) reagent. After washing in water the reaction product was darkened by treatment with 0.5% copper sulphate (W/v in saline) for 5 min. Sections were then counterstained with Mayer's haematoxylin and mounted in DPX. 0.05 M Tris-HCl (pH 7.6) buffered saline was used for all reagant dilutions and washes. This enhanced, triple layer, double conjugate method was essential for adequate detection of CD25$^+$ (OX-39$^+$) inflammatory cells which by conventional two layer indirect methods were only poorly stained. Negative staining controls consisted of omission of the primary or secondary antibody, application of the DAB reagent alone and substitution of the primary antibody with normal mouse serum or monoclonal antibodies to human antigens (e.g. OKT 6; Ortho Diagnostics).

**Table 1.** Monoclonal antibodies to rat antigens: dilutions and specificities

| Antibody[a] | Dilution[b] | Specificity |
|---|---|---|
| MRC OX-6 | 1:1000 | I-A (monomorphic), macrophages, B cells, dendritic cells |
| MRC OX-17 | 1:1000 | I-E (monomorphic), macrophages, B cells, dendritic cells |
| MRC OX-1 | 1:1000 | Leukocyte common antigen |
| MRC OX-8 | 1:1000 | CD8, thymocytes, Ts/c, NK cells |
| MRC OX-12 | 1:1000 | kappa chains, B cells |
| MRC OX-19 | 1:1000 | CD3, thymocytes, pan T |
| MRC OX-39 | 1:500 | CD25, Il-2r, activated T cells and macrophages, NK cells and some B cells |

[a] All monoclonal antibodies were purchased from Serotec
[b] Dilutions found to give optimal staining after checkerboard titration

*Evaluation of tissue sections.* The presence or absence of epithelial Ia$^+$ (I-A & I-E) cell foci was assessed by examination of three pairs of 5 μm sections, at 50 μm intervals, from each tissue block stained with OX-6 and OX-17 monoclonal antibodies. A focus was defined as a collection of 10 or more Ia$^+$ epithelial cells.

Estimation of the density of CD25$^+$ cells associated with Ia$^+$ or Ia$^-$ infiltrating carcinomas and adjacent dysplastic epithelium was performed by examination of three pairs of serial sections taken at 50 μm intervals from each specimen (Group II rats). One section from each pair was stained for Ia (I-A; OK-6) and the other for CD25 (OX-39). CD25$^+$ cell counts were performed using a ×40 objective and ×10 oculars incorporating a 10×10 mm ocular grid. CD25$^+$ cells within 325 μm of the basement membrane of Ia$^+$ or Ia$^-$ epithelium, determined by examination of the serial sections stained for Ia, were counted and the area determined using a Leitz Imigan 2 image analyser. Results are expressed as the number of cells/mm$^2$.

Determination of the number of Ia$^+$ (I-A, OX-6) epithelial cell foci, defined as 10 or more positive cells, and its relationship to Il-2r bearing cells was performed on three pairs of serial sections taken at 50 μm intervals from the lingual specimens of Group III rats. The number of Ia$^+$ epithelial cell foci present in the dorsal lingual epithelium was counted at a magnification of ×160. CD25$^+$ cells, within the epithelium and down to underlying muscle, were counted at a magnification of ×400 throughout the whole length of the dorsal lingual epithelium.

**Table 2.** Incidence of focal epithelial Ia reactivity in non-invasive epithelium of rats painted with 4NQO (3 × weekly) for up to 9 months

| Months | No. of 4NQO applications | Nr. rats /group | No. of rats showing $Ia^+$ epithelium: | | |
|---|---|---|---|---|---|
| | | | Palatal | Lingual | Palatal and/or lingual |
| 1 | 12 | 5 | 4 | 5 | 5 |
| 2 | 24 | 6 | 2 | 3 | 4 |
| 3 | 36 | 6 | 2 | 2 | 3 |
| 4 | 48 | 6 | 1 | 2 | 2 |
| 5 | 60 | 6 | 3 | 4 | 4 |
| 6 | 72 | 6 | 2 | 2 | 2 |
| 7 | 84 | 6 | 1 | 1 | 2 |
| 8 | 96 | 5 | 2 | 1 | 3 |
| 9 | 108 | 5 | 2 | 1 | 2 |

The total length of dorsal epithelium and area in which $CD25^+$ cells were found was determined using a Leitz Imigan 2 image analyser and the results expressed as $Ia^+$ foci/mm or cells/$mm^2$. In 4 specimens where confluent expression of Ia was found throughout the entire dorsal epithelium an arbitrary score of 10 foci/mm was allocated.

Subjective assessment of the presence and relative proportions of different cell types was performed on serial sections stained with the different monoclonal antibodies (Table 3).

*Statistic.* Statistical analyses were performed using the Mann Whitney test.

## Results

### *Prolonged 4NQO-treated epithelium*

Ia reactivity in non-invasive palatal and lingual epithelia after prolonged exposure to 4NQO was usu-

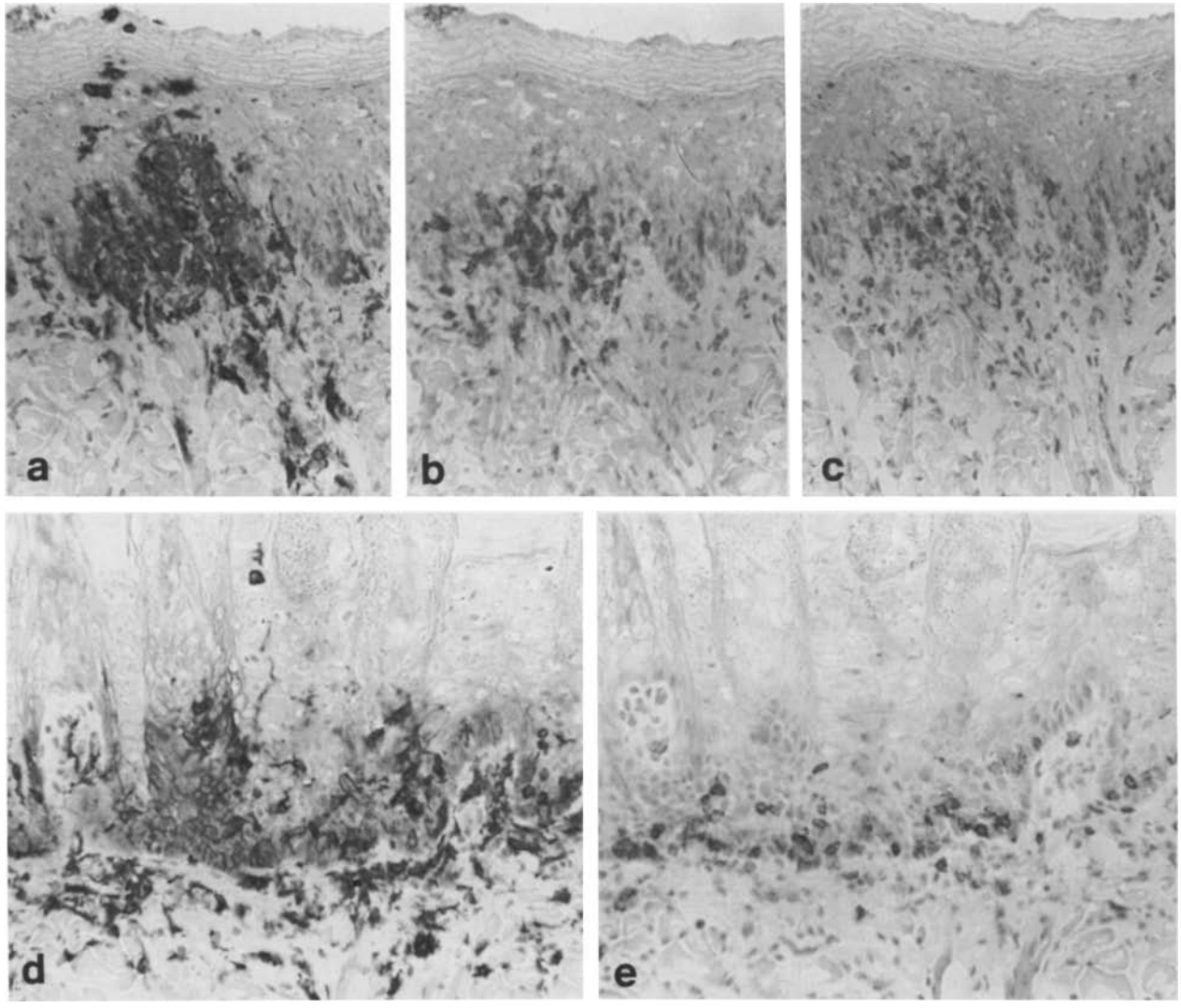


**Fig. 1 a–c.** Focal expression of Ia by lingual epithelium **a** associated with the local presence of $OX\text{-}19^+$ T cells **b** and $CD25^+$ cells **c**. The more generalised pattern of epithelial Ia reactivity **d** is also associated with $CD25^+$ cells **e**. Magnification × 250

**Table 3.** Summary of the inflammatory cell infiltrate associated with rat lingual squamous cell carcinomas induced by 4NQO

| Carcinoma number | Epithelial Ia expression[a] | | Inflammatory cell infiltrate[b] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Infiltrating | Overlying | OX-1 | OX-19 | OX-6 | OX-8 | OX-39 | OX-12 |
| 1 | − | ± | + | + | +++ | ± | ± | − |
| 2 | − | ± | + | + | ++ | ± | ± | − |
| 3 | + | ± | ++ | + | +++ | ± | ± | − |
| 4 | − | − | ++ | ± | ++ | + | ± | − |
| 5 | − | − | ++ | + | ++ | − | − | − |
| 6 | +++ | − | +++ | ++ | +++ | ++ | ++ | − |
| 7 | − | + | ++ | + | +++ | − | ± | − |
| 8 | ++ | − | +++ | ++ | +++ | ++ | + | − |
| 9 | +++ | ++ | ++ | ++ | +++ | + | + | − |
| 10 | − | − | ++ | + | +++ | ++ | − | − |
| 11 | ++ | ++ | +++ | + | +++ | ++ | + | − |
| 12 | +++ | ++ | +++ | ++ | +++ | ++ | + | − |

[a] −, no; ±, poor; +, weak; ++, moderate and +++, strong staining for Ia
[b] −, no; ±, sparse; +, small; ++, moderate and +++, large numbers of positive cells. Scored to allow comparisons between specimens (OX-1) or within specimen (OX-19, 6, 8, 12 and 39)

ally focal in nature (Fig. 1a). After 1 month 100% of rats expressed epithelial Ia in the tongue, but, thereafter (2–9 months 4NQO treatment) epithelial Ia expression was present in only 20–40% of animals in both the tongue and the palate (Table 2). In all cases there was concordant expression of both I-A and I-E as assessed by reactivity with OX-6 and OX-17 antibodies respectively. Both focal and the rare instances of more generalised epithelial Ia expression were associated with an inflammatory infiltrate beneath and within the epithelium containing T cells and $CD25^+$ cells (Fig. 1). The majority of $CD25^+$ cells appeared to be small lymphocytes. B cells, determined by staining for surface membrane kappa chain, were absent and few cells conforming to an NK cell phenotype (large granular $OX\text{-}19^-$, $OX\text{-}1^+$, $OX\text{-}8^+$ cells) were present within inflammatory infiltrates associated with $Ia^+$ epithelial cells.

*Lingual squamous cell carcinomas*

The expression of epithelial Ia and the nature of the underlying inflammatory cell infiltrate adjacent to the lingual carcinomas are shown in Table 3. The infiltrating epithelium in 6 of the 12 carcinomas showed a generalised cell surface and cytoplasmic pattern of Ia expression, but Ia in the dysplastic overlying epithelium did not always correspond to that observed in the infiltrating epithelium. The inflammatory cell infiltrate consisted predominantly of $Ia^+$ cells, T cells ($OX\text{-}19^+$), some NK cells (large granular $OX\text{-}19^-$, $OX\text{-}1^+$, $OX\text{-}8^+$ cells) and $CD25^+$ ($OX\text{-}39^+$) cells in variable proportions. NK cells were located at a distance from the malignant epithelium, a feature which contrasted with T cells and $CD25^+$ cells which appeared to be concentrated in areas close to epithelial Ia reactivity.

Cell counts demonstrated that there were significantly more $CD25^+$ cells closely associated with $Ia^+$ epithelium compared to $Ia^-$ epithelium (Fig. 2) in both the infiltrating epithelium ($p < 0.005$) and the dysplastic epithelium overlying/adjacent to the carcinomas ($p < 0.001$). Furthermore, significantly more $CD25^+$ cells were found in association with the infiltrating epithelium than the overlying/adjacent epithelium and this was evident in areas of both $Ia^+$ ($p < 0.030$) and $Ia^-$ ($p < 0.02$) epithelial staining.

*Development of epithelial Ia reactivity*

The number of rats with focal Ia reactivity in the palatal and lingual epithelia after painting with 4NQO for 28 days is shown in Table 4. Epithelial $Ia^+$ foci became evident after 7–12 days of 4NQO treatment and were present in the lingual epithelia of all rats at 28 days. In this particular experiment the expression of Ia in palatal epithelium was less frequent with only 2 of 5 rats showing focal Ia after 28 days 4NQO treatment. This difference was not considered significant as Group I animals and our previous results (Matthews et al. 1986) did not show this trend. The relationship between $CD25^+$ cells and epithelial Ia expression, therefore, was examined in the lingual mucosa.

The expression of Ia by keratinocytes in lingual epithelium painted with 4NQO for up to 28 days

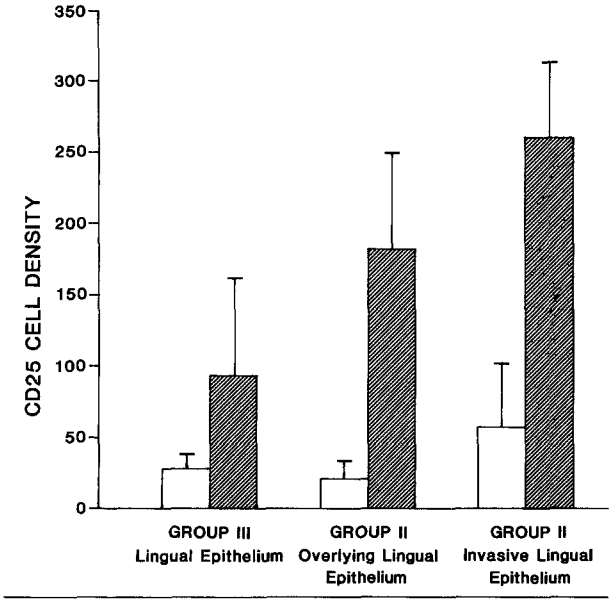


**Fig. 2.** $CD25^+$ cell densities ($\pm$1SD) associated with lingual specimens exhibiting areas of $Ia^+$ or $Ia^-$ epithelium. Mean areas ($mm^2$)/specimen counted to determine cell densities: (a) Group III; $Ia^-$, 4.1; $Ia^+$, 4.0; (b) Group II overlying epithelium; $Ia^-$, 2.0; $Ia^+$, 0.7; (c) Group II invasive; $Ia^-$, 1.0; $Ia^+$, 1.0. It should be noted that Group III results are based on counts throughout whole $Ia^+$ or $Ia^-$ specimens and not, as in Group II, based on comparing $Ia^+$ and $Ia^-$ areas within a specimen (see Methods). In this latter case areas counted vary according to the amount of overlying/invasive epithelium present and extent of Ia expression

**Table 4.** Incidence of focal epithelial Ia reactivity in non-invasive epithelium of rats painted with 4NQO (3 × weekly) for up to 28 days (5 rats/group)

| Days | No. of 4NQO applications | No. of rats showing $Ia^+$ epithelium: | | |
|---|---|---|---|---|
| | | Palatal | Lingual | Palatal and/or lingual |
| 3 | 1 | 0 | 0 | 0 |
| 5 | 2 | 0 | 0 | 0 |
| 7 | 3 | 0 | 1 | 1 |
| 10 | 4 | 0 | 1 | 1 |
| 12 | 5 | 1 | 4 | 4 |
| 14 | 6 | 1 | 1 | 1 |
| 17 | 7 | 2 | 2 | 2 |
| 19 | 8 | 2 | 3 | 3 |
| 21 | 9 | 0 | 2 | 2 |
| 24 | 10 | 2 | 1 | 3 |
| 26 | 11 | 3 | 4 | 4 |
| 28 | 12 | 2 | 5 | 5 |

correlated closely with the density of $CD25^+$ cells in the inflammatory cell infiltrate (Fig. 3). There was no indication that $CD25^+$ cell density increased before the appearance of $Ia^+$ EC foci or

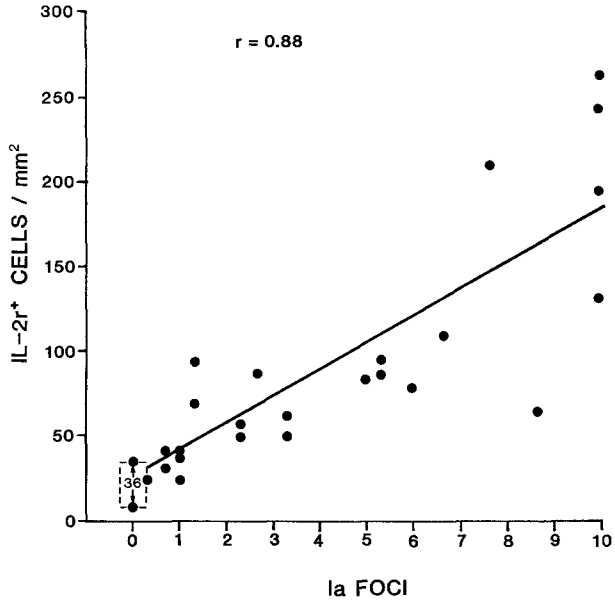


**Fig. 3.** Scattergam and regression line ($r = 0.88$; $p < 0.001$) showing the relationship between $CD25^+$ cell density and number of $Ia^+$ epithelial cell foci/mm lingual surface in Group III animals. 36 specimens exhibited no epithelial expression of Ia and fell within the *dotted area*

vice versa and apparent peaks of $Ia^+$ EC foci and $CD25^+$ cells on days 12, 19 and 26 were not significant (data not shown). Furthermore, the density of $CD25^+$ cells in Group III rats with $Ia^+$ lingual epithelium were significantly higher ($p < 0.001$) than those found in rats with $Ia^-$ lingual epithelium (Fig. 2).

### *Controls*

Foci of $Ia^+$ keratinocytes were not evident in any of the solvent-only treated control rats. The staining controls used to examine the specificity of the anti-Ia antibody reactivity were all negative.

## Discussion

The results of this study demonstrate that treatment of rat oral mucosa with the carcinogen 4NQO leads to the induction of epithelial Ia expression. Although epithelial Ia reactivity has been reported in a variety of different tumours and tumour cell lines (Carrel et al. 1986) this is the first time that the incidence of epithelial Ia reactivity has been studied during tumour development thus allowing comparison between normal, dysplastic and malignant tissues. Our results indicate that, in this carcinogenesis model, there is a common mechanism leading to induction of epithelial Ia as all rats at one month and 30–50% of animals ther-

after expressed oral epithelial Ia. Furthermore both class II products, I-A and I-E, showed similar patterns of localisation and epithelial expression agreeing with studies of other rodent models (Mayrhofer et al. 1983). By contrast studies in man have usually demonstrated non-coordinate expression of HLA-D region products suggesting independent D gene regulation (Basham et al. 1985; Moore et al. 1986; Savage et al. 1987; Gawkrodger et al. 1987).

The stimuli for epithelial Ia expression in the majority of physiological and pathological situations are largely unknown although hormones (Klareskog et al. 1980), plant lectins (Pujol-Borrell et al. 1983), antigenic stimulation (Barclay & Mason 1982; Scheynius and Tjernlund 1985) and IFN-$\gamma$ (Basham et al., 1984, 1985; Schwartz et al., 1985; Volc-Platzer et al., 1985) have all been implicated. While the inflammatory cell infiltrate induced by 4NQO and present throughout tumour development consisted of a mixed population of macrophages, NK cells and T cells, in agreement with our previous studies (Matthews et al. 1986), the present data demonstrate that epithelial Ia expression is closely associated with the local presence of $CD25^+$ cells. Receptors for Il-2 are expressed on activated T cells (Waldmann 1986) and macrophages (Hancock et al. 1987), NK cells (Yodoi et al. 1985) and transformed B cells (Zubler et al. 1984). In this and our previous study (Matthews et al. 1986) we have demonstrated the absence of B cells in the inflammatory infiltrate, that NK cells when present are found distant from tumour islands ($Ia^+$ or $Ia^-$) and that macrophage-like cells ($Ia^+$, OX-$1^+$, W3/$25^+$, OX-$19^-$, OX-$8^-$) are the predominant cell type in all infiltrates irrespective of epithelial Ia status. From these considerations and the subjective observation that in many cases T cells were concentrated close to $Ia^+$ epithelium (Fig. 1) it is probable that the $CD25^+$ cells represent activated T cells. To date we have been unable to directly characterise the $CD25^+$ cell population by double labelling methods because of the relatively low level of expression of Il-2r by the cells concerned (data not shown).

Although previous attempts to identify a particular cell population associated with epithelial expression of Ia have been unsuccessful (Whitwell et al. 1984; Moore and Ghosh 1987) a recent report has demonstrated that HLA-DR expression by gut epithelium in ulcerative colitis and Crohns disease is related to the presence of $CD25^+$ cells in the lamina propria (Fais et al. 1987). Our study supports and extends this report by demonstrating a correlation between $CD25^+$ cells in the inflammatory infiltrate and the class II antigen status of associated oral epithelial tissue (normal, dysplastic and malignant). Such a correlation is consistent with the concept that IFN-$\gamma$, locally synthesised and secreted by $CD25^+$ cells, is the stimulus for epithelial Ia expression. This is supported by in vitro studies showing that IFN-$\gamma$ induces expression of class II antigen by epidermal cells (Czernielewski and Bagot 1986; Basham et al. 1984, 1985). However, our in situ studies do not prove a causal relationship nor do they indicate a particular sequence of events as we failed to demonstrate an increase in $CD25^+$ cell densities prior to epithelial expression of Ia or vice versa. Furthermore we have shown increased Ia expression by the six cell lines established to data from 4-NQO rat tumours after 3 days culture with 350 U/ml IFN-$\gamma$ (Crane et al. 1988). Interestingly three of the six cell lines showed a low level of Ia expression in long term culture in the absence of IFN-$\gamma$. This data together with that of Moore et al (1986), showing that expression of class II antigen by colorectal carcinoma cells cannot by correlated with presence of $CD25^+$ cells or induced by IFN-$\gamma$, suggests that factors other than IFN-$\gamma$ are necessary in some circumstances. Indeed initiation of class II antigen expression by epithelial cells due to unknown agents (eg. viruses) has been suggested as the primary event in certain autoimmune diseases (Todd et al 1986). In such circumstances a local autoimmune response to epithelium is provoked causing tissue damage which, even if the initiating agent is removed, may be chronically stimulated by continued class II antigen expression induced by locally produced immune factors (IFN-$\gamma$).

The physiological role of Ia expression by epithelial cells remains obscure (Unanue and Allen 1986). If Ia expression is a general feature of epithelial cells its physiological function, if any, is unlikely to be that of stimulating an autoimmune response. Furthermore a role in antigen presentation for stimulating positive responses to foreign antigens appears unnecessary particularly in sites such as epidermis and oral epithelium which possess functional Langerhans cell populations. However recent in vitro studies examining the stimulatory role of HLA-$DR^+$ keratinocytes (in mixed skin-lymphocyte reactions; Czernielewski 1985) and $Ia^+$ gut epithelial cells (in specific antigen stimulation studies; Bland and Warren 1987a, 1987b) suggest that the physiological role of epithelial Ia expression may be to present antigen in a form which downregulates local immune reactions. Thus uncontrolled immune reactions (eg. due to chronic antigenic challenge) might be locally depressed by

antigen associated with $Ia^+$ epithelium. Hence epithelial expression of Ia, constitutively expressed or induced by local factors such as IFN-$\gamma$, might serve a protective role by limiting host tissue damage and/or presenting altered self antigens in a form which downregulates potential autoreactive lymphocytes. Indeed there is evidence that MHC antigen expression by thymic epithelium, which is independent of $CD25^+$ cells, can regulate T cell development and specificity (Kappler and Marrack 1978; Owen et al. 1986). Such a downregulatory function could have important consequences in the behaviour of epithelial neoplasms. For example, $Ia^+$ epithelial tumours might depress any anti-tumour antigen response and thus escape immune surveillence; a thesis with some experimental support (Taramelli et al. 1984). Whether the decline in epithelial expression of Ia and associated reduced numbers of $CD25^+$ cells after the first month of carcinogen treatment and the observed high incidence of tumours at nine months are consequences of an early downregulatory event remains to be tested. To date results from studies investigating the relationship between tumourogenicity of 4NQO induced oral carcinoma cell lines and MHC expression has failed to show any correlation (Crane et al. 1987). However as these findings were dependent upon assessment of tumourigenicity by growth in agar or nude mice, rather than immunocompetent hosts, further work is required to investigate the extent to which class II antigen status may modify the biological behaviour of epithelial neoplasms.

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