

The Basement Membrane in Experimentally Induced Atypias and Carcinoma of the Uterine Cervix in Mice

An Immunofluorescence Study

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Summary. Basement membrane-specific antigens of the squamous epithelium of the uterine cervix were investigated in 19 normal mice, in 7 mice with cervical atypia and in 3 mice with invasive carcinoma. Cervical atypia and carcinoma were induced by local application of benzo(a)pyrene. Basement membrane-specific antigens were demonstrated by immunofluorescence with sera from patients with bullous pemphigoid. Both normal squamous cervical epithelium and atypical cervical epithelium showed the presence of a continuous, clearly delineated basement membrane. Clusters of invasive squamous carcinoma were also surrounded by a fluorescent basement membrane which, however, appear fragmented or discontinuous. The results suggest that the ability of cervical squamous cells to secrete basement membrane antigens is not completely lost during carcinogenesis, thus substantiating our previous observations in the cervix of human subjects.

Key words: Cervical carcinogenesis – Basement membrane – Immunofluorescence.

Introduction

Beutner et al. (1968) demonstrated the existence of a continuous basement membrane beneath several squamous epithelia including those of the oesophagus, vagina and skin. These authors showed that antibodies in sera from patients with bullous pemphigoid reacted specifically with antigens of the basement membrane. Using Beutner's technique we demonstrated that there is, in the human uterine cervix, a continuous basement membrane, not only beneath normal squamous epithelium but also deep to epithelium with dysplasia and

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carcinoma in situ (Rubio and Biberfeld, 1975). In a previous study we have observed deviations from the normal appearance in the basement membrane of invasive squamous carcinoma of the human cervix (Rubio et al., 1978).

Preliminary experiments at this laboratory indicated that sera from patients with bullous pemphigoid also reacted with the basement membrane present in the normal squamous epithelium in the cervix of mice. These observations prompted us to investigate the characteristics of the basement membrane under controlled conditions of induced cervical carcinogenesis in mice.

Material and Methods

Twenty-five virgin C57B1 mice were painted intravaginally twice a week with 1% benzo(a)pyrene in acetone as recommended by Koprowska (1958) for five months. Seven control animals were treated with acetone alone in parallel. After this time the cervix-portion area was removed and stored at -78°C .

Immunofluorescence (IF)

Sera from patients with bullous pemphigoid and with high titres of anti-basal membrane antibodies (BM) were selected for this study.

As previously described by Beutner et al. (1968) we used patients with pemphigus to demonstrate intercellular antigens (IC) of the cervical squamous epithelium by IF. Stromal reticulin in the connective tissue was investigated also by IF, using sera obtained from patients with the malabsorption syndrome (Alp and Wright, 1971), containing antibodies to reticulin (courtesy of Dr. A. Fagraeus). All sera were kept frozen (-70°C) until the cervical tissues were examined.

Five μm thick frozen sections from the cervix were stained by an indirect immunofluorescent technique (Biberfeld et al., 1975). Parallel frozen sections were stained for conventional histological studies. Our criteria for cervical atypia and for squamous carcinoma in mice have been previously described (Rubio and Lagerlöf, 1974).

Results

Nineteen of the 32 animals investigated showed *normal epithelium* (12 animals had been treated with benzo(a)pyrene and 7 animals with acetone alone). The immunofluorescence studies demonstrated the presence of a continuous, clearly delineated basement membrane beneath the normal squamous epithelium of the cervix (Fig. 1).

Of the remaining 13 animals, ten showed intraepithelial atypia and three invasive carcinoma. The *atypical epithelium* showed a clearly delineated BM (Fig. 2), closely resembling that seen deep to normal epithelium.

Studies with AR sera in animals with normal and atypical epithelium demonstrated reticulin fibres scattered randomly in the connective tissue but no apparent condensation of such fibres in the subepithelial area (Fig. 3).

In the three mice with *invasive squamous carcinoma*, invading cell clusters were observed to be surrounded in part by a fluorescent basement membrane. This membrane was fragmented or discontinuous (Figs. 4, 5). Invasive carcinoma

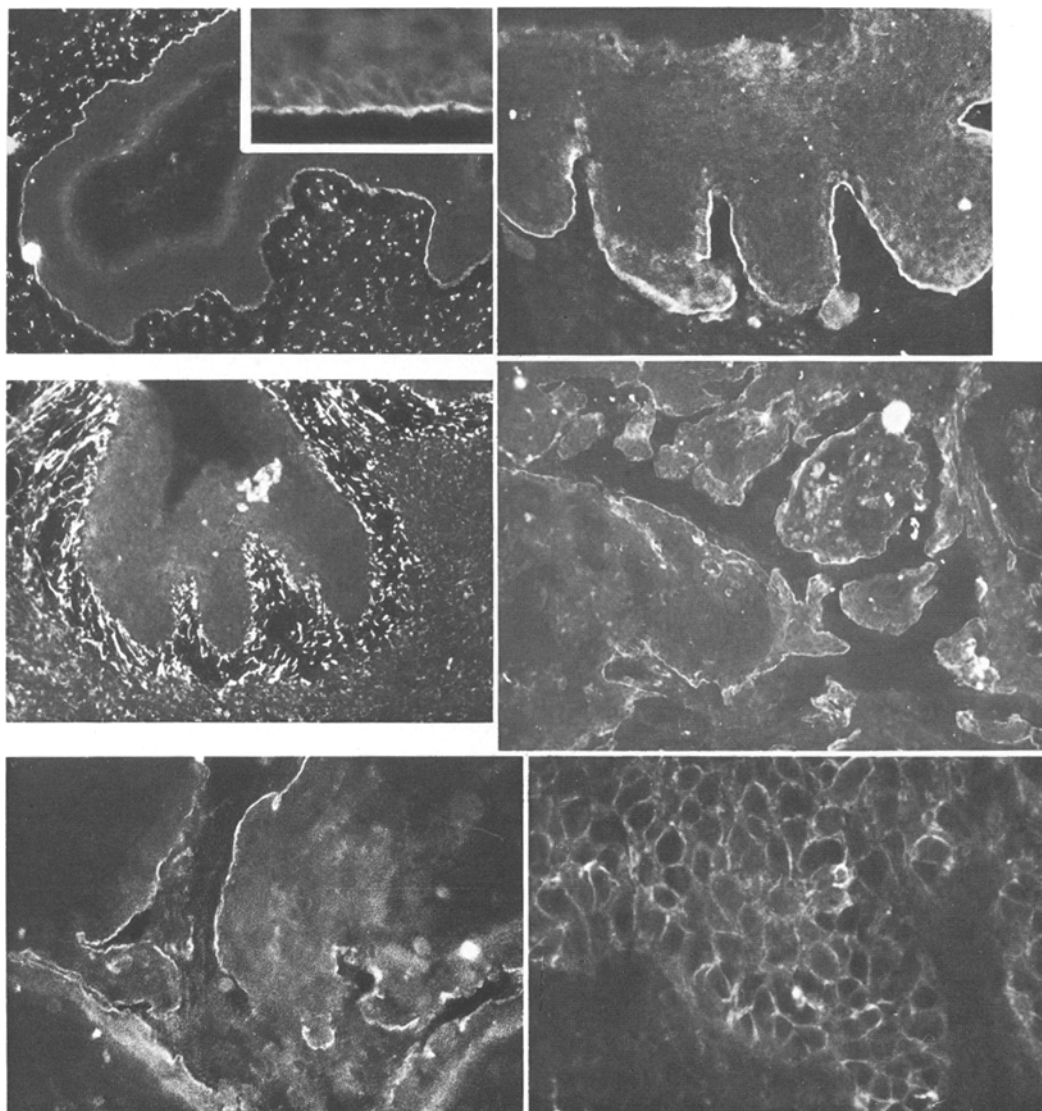


Fig. 1. Immunofluorescent, continuous basement membrane beneath the normal cervical epithelium ($\times 60$). Insert: High power view of normal basement membrane ($\times 250$)

Fig. 2. Immunofluorescent, continuous basement membrane beneath cervical epithelium with atypia ($\times 80$)

Fig. 3. Immunofluorescent reticulin in the stroma surrounding atypical cervical epithelium. Note lack of continuous fluorescent substance along the epithelial-stroma interface ($\times 60$)

Fig. 4. Invasive squamous carcinoma of the cervix showing discontinuous basement membrane ($\times 60$)

Fig. 5. Detail of Fig. 4. Compare the quality of the basement membrane surrounding invading clusters with the insert in Fig. 1 ($\times 250$)

Fig. 6. Clusters of invasive squamous carcinoma of the cervix showing distinct immunofluorescent intercellular substance ($\times 400$)

challenged with AR serum showed no particular condensation of fluorescent substance surrounding invading cell clusters.

Normal cervical epithelium, atypical epithelium and invasive carcinoma examined with anti-IC antibodies showed a similar net-like fluorescent pattern surrounding individual cells (Fig. 6).

Discussion

Invasive squamous carcinoma of the cervix in mice is preceded by histologically detectable alterations known collectively as intraepithelial atypias (Koprowska et al., 1958; Rubio, 1977). Similar epithelial alterations occur in human beings preceding invasive carcinoma. The penetration of connective tissues by cancer cells is definitive histological evidence of tumor invasion and according to many authors (Foushee et al., 1969; Marcuse, 1971) the penetration of malignant cells is preceded by the disruption of the basement membrane.

The basement membrane has been considered to be a condensation of reticulin fibres beneath the epithelium (Younes et al., 1965), but the demonstration of such a continuous condensation of reticulin fibres is difficult to evidence by conventional histological techniques (Lamb et al., 1960). However, Beutner's studies (1968) have demonstrated the presence of particular antigens in a basement membrane-like pattern beneath the squamous epithelium, which are defined by their reaction with antibodies occurring in patients with bullous pemphigoid. Using this method we have shown a continuous immunofluorescent basement membrane underneath both normal and atypical squamous epithelium of the cervix, both in mice and in human subjects (Rubio and Biberfeld, 1975). These observations are in contradiction to the views of Lamb (1960) and Hamperl (1965). Those authors found in conventional histological preparations of the cervix that the basement membrane of the normal squamous epithelium is often disrupted. Our results, however, are in agreement with those reported by Luibel et al. (1960) and Ashworth et al. (1961) based on electron microscopical studies of the human cervix. These authors found that the basement membrane in normal epithelium and in carcinoma in situ was complete and intact, but often showed marked alterations in invasive carcinoma. Frithiof (1972), using electron microscopy also found in invasive squamous carcinoma of the oral cavity clusters surrounded by extremely thin or multilayered basement membrane alternating with absence of such a membrane.

From our observations it appears that the ability to produce and deposit basement membrane antigens beneath the squamous epithelium of the uterine cervix in mice is – at least partly – retained during all phases of carcinogenesis. These results, in experimental animals, substantiate our previous observations in the cervix of human subjects.

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