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HLA molecule expression in cutaneous squamous cell carcinomas: an immunopathological study and clinical-immunohistopathological correlations

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Summary. A correlation between the lack of MHC class I gene expression on murine tumor cells and their ability to grow and metastasize has recently been established. This paper studies HLA-ABC and HLA-DR antigen expression in tumor cells and mononuclear infiltrate of 26 cutaneous squamous cell carcinomas (SCC).

Our results showed a heterogeneous expression of HLA class I molecules in these tumors. No significant correlation between the degree of HLA class I molecule expression and anatomical-clinical parameters was found. Class II antigen expression was correlated to the histopathological type and to the degree of cell differentiation. Most mononuclear cells infiltrating the tumor were T-lymphocytes. No correlation with anatomical-clinical parameters was found.

Key words: Cutaneous squamous cell carcinomas, HLA-ABC, HLA-DR

Introduction

Human histocompatibility antigens (HLA) are highly polymorphic glycoproteins encoded by closely-linked loci on chromosome 6. HLA class I antigens are expressed on most nucleated cells, whereas HLA class II antigens show a restricted expression pattern (Murphy et al., 1981; Hirschberg et al., 1982). Class I antigens are important recognition structures in immune response. Reduced or abolished expression of HLA class I antigens has been reported on several human neoplasias (Natali et al., 1983, 1984; Ruiter et al., 1984; Hua et al., 1985; Momburg et al., 1986, 1989; García-Espejo et al., 1986). Using different murine tumor models, it has been shown that malignant capacity is correlated with the loss of class I histocompatibility molecule expression (Festenstein et al., 1982; Schmith and Festenstein, 1982). Furthermore, when these cells are transfected with H-2 class I genes, the subsequent surface expression of these molecules abrogate their invasive capacity (Hui et al., 1984; Tanaka et al., 1985; Wallich et al., 1985).

Class II antigens are important for communication, interaction, and regulation of cell systems colaborating in the induction of the immune response. The expression of HLA-DR is important as a restriction element for the induction and proliferation of the helper T-lymphocytes (Quigstad et al., 1983; Niedecken et al., 1988).

The importance of HLA antigen expression on solid tumors in the interactions with autologous T-lymphocytes was recently demonstrated by Vánky et al. (1987, 1988, 1989). In another study by Natali et al. (1981) which examined cell suspension and cryostat sections from malignant melanoma, they found heterogeneous HLA-DR expression, with a variable proportion of negative cells in all their cases.

Human SCC are characterized by a high metastatic potential. It has been postulated that mononuclear cell infiltration surrounding tumor masses plays a role in an immunological reaction against the tumor and aggressive biological behaviour appears to be related to defects in the immunological status or in local immunosurveillance (Murphy et al., 1982). It has been found that mononuclear cell infiltrates consist mainly of T- and B-cell activates, Langerhans cells (LC) and monocytes (Habets et al., 1989).

The mechanism responsible for the expression of class II antigens on keratinocytes in the lesions of these cutaneous tumors is still debatable (Chen et al., 1988). Considering the role of histocompatibility antigens in the recognition of tumor cells by T-lymphocytes, the aim of this paper is to further investigate HLA antigen expression on different types of SCC and the relationship that it has on the infiltrating lymphocyte activation markers and on the clinical-pathological criteria of malignancy.

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Materials and methods

Patients

Twenty-six primary cutaneous SCC obtained from patients, who at the time of excision were not being treated with chemotherapy and/or radiotherapy were studied. Detailed clinical questionnaires were maintained and included information relevant to skin type, sun exposure and biological aggressiveness of lesions (size, ulceration, rapidity of growth) and tumor-associated cutaneous lesions. There were no signs suggesting immunological abnormalities or metastatic lesions. Upon removal, tumor specimens were divided into two parts: one was fixed in 10% buffered formaldehyde and processed for histological examination, whereas the other was embedded in Tissue-Tek II O.C.T. compound (Milles Laboratories), frozen immediately in liquid nitrogen and stored at -80 °C until used. The clinical diagnosis of SCC was confirmed in all cases by histological examination of paraffin-embedded sections stained with haematoxylin-eosin. The degree of tumor cell differentiation, tumor invasion and mononuclear cell infiltration were also evaluated (Table 1).

Immunohistochemical staining

Serial cryostat sections (6 μm thick) were air-dried, fixed in acetone for 10 minutes and stained by the indirect immunoperoxidase technique. Briefly, after washing with phosphate-buffered saline (PBS, pH 7.4), endogenous peroxidase was blocked by incubation with hydrogen peroxide (3%) for 10 min. Sections were then extensively washed in PBS and treated with normal swine serum diluted to 1:20 in 0.05M Tris buffer (pH 7.6) for 20 min. The specimens were incubated on slides with monoclonal antibodies (MoAb), obtained in mouse, as indicated in Table 2 at the optimal dilution and incubated for 20 min. They were then sequentially incubated with peroxidase, labelled rabbit antimouse immunoglobulin (20 min) and peroxidase, labelled swine antirabbit immunoglobulin (20 min). Washings between incubations were performed with PBS and incubations were performed in a humidified chamber at room temperature. The reaction was revealed by incubation with 0.05% 3-amino-9-ethylcarbazole (10 min) and 0.01% hydrogen peroxide in 0.1M acetate buffer (pH 5.2). The slides were rinsed in distilled water, counterstained with haematoxylin and mounted with glycerol gelatin. Negative controls without primary monoclonal antibody and normal mouse serum were included. All reagents were from Dako corporations.

Observations were carried out in the tumor masses, in the cell infiltrates surrounding SCC and in adjacent skin.

Statistical analysis

The relationship between HLA molecule expression on tumor cells and clinical-pathological parameters was assessed with the chi square test.

Table 1. Histopathological characteristics of 26 cutaneous S.C.C. studied.

CASE	CELL DIFFERENTIATION DEGREE	TUMOR INVASION	HISTOPATHOLOGICAL CLASSIFICATION
1	Good	Superficial dermis	Type I
2	Moderate	Profound dermis	Type III
3	Good	Superficial dermis	Type II
4	Good	Superficial dermis	Type II
5	Poor	Profound dermis	Type IV
6	Good	Profound dermis	Type I
7	Good	Profound dermis	Type I
8	Moderate	Profound invasion	Type III
9	Moderate	Profound dermis	Type IV
10	Good	Superficial dermis	Type I
11	Good	Superficial dermis	Type I
12	Moderate	Profound dermis	Type II
13	Good	Profound dermis	Type I
14	Good	Superficial dermis	Type I
15	Good	Superficial dermis	Type II
16	Good	Profound dermis	Type I
17	Good	Superficial dermis	Type I
18	Good	Superficial dermis	Type I
19	Good	Superficial dermis	Type I
20	Good	Superficial dermis	Type I
21	Moderate	Profound dermis	Type III
22	Moderate	Subcutaneous	Type II
23	Good	Superficial dermis	Type I
24	Moderate	Subcutaneous	Type II
25	Poor	Profound invasion	Type III
26	Poor	Profound dermis	Type III

Results

Most of the SCC studied were located in sun-exposed skin.

HLA class I antigen expression on tumor cells

The monoclonal antibody, W6/32 was used to stain the normal epidermal cells from the basal to the granular cell layers and served as a positive control. The tumor cell expression pattern with anti HLA-ABC MoAb was very heterogeneous; 30% exhibited uniform superficial staining of most tumor cells while 35% showed scattered

Table 2. Monoclonal antibodies.

	SPECIFICITY	DILUTION
W6/32 (ATCC)	HLA-A, B, C	1/1,000
EDU (Vilella, R.)	HLA-DP, DQ, DR	1/100
OKT3 (Ortho)	CD-3	1/1,000
OKT6 (Ortho)	CD-1a	1/50

Table 3. HLA antigen expression on cutaneous squamous cell carcinomas and their mononuclear infiltrates.

	TUMOR PERCENTAGES (staining pattern)*		
	+	±	
Tumor			
HLA class I (W6/32)	30	35	35
HLA class II (EDU-1)	8	35	57
Infiltrate			
HLA class II (EDU-1)	43	13	44

(*). +: homogeneous positive; ±: partially positive; -: negative.

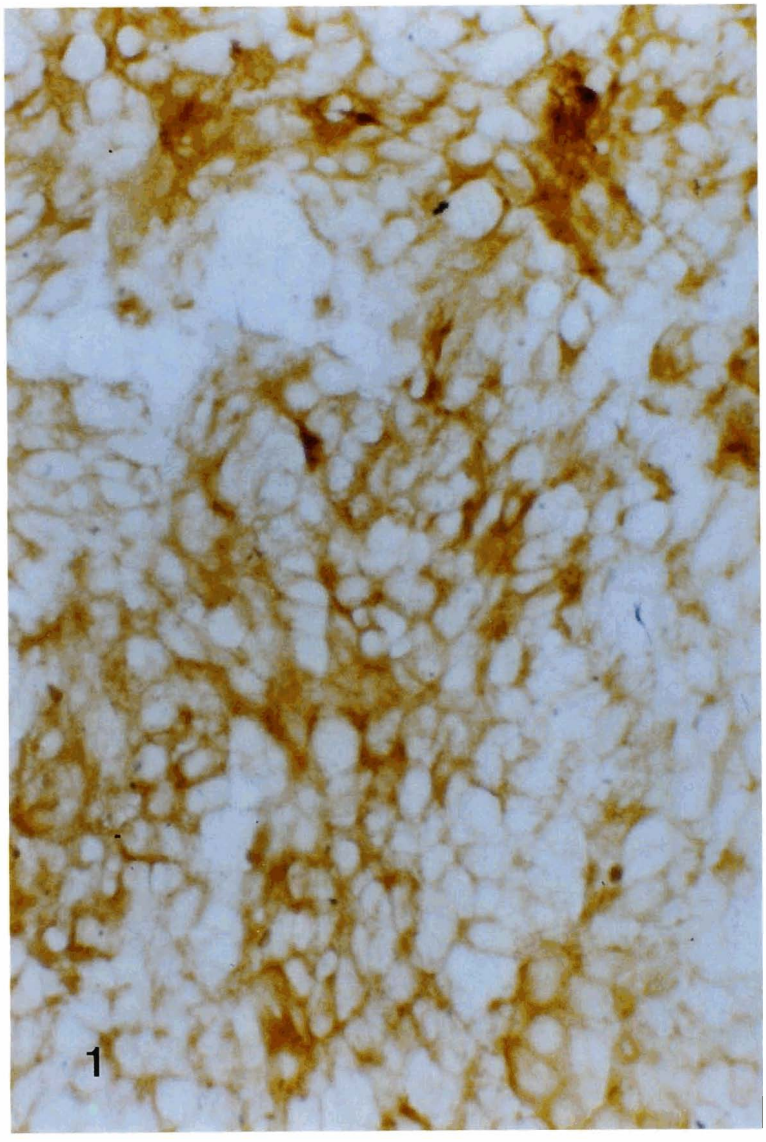

Fig. 1. Squamous cell carcinoma (without counterstain) showing HLA class I antigens. Staining W6/32 MoAb. x 2,000

staining in only a few areas of the tumor (Fig. 1). The remaining 35% showed no significant staining for HLA-ABC MoAb. These results are summarized in Table 3. No correlation was found between HLA class I antigen expression and the clinical pathological parameters (Table 4) or between HLA class II expression on tumor cells or infiltrating lymphocytes (Table 5).

Table 4. Statistical correlation between HLA expression and histopathological tumor characteristic of cutaneous S.C.C.

	HISTOPATHOLOGICAL TYPE		CELL DIFFERENTIATION		TUMOR INVASION	
	X^2	p	X^2	p	X^2	p
Tumor HLA class I	3.73	n,s,	6.13	n.s.	9.58	n.s.
Tumor HLA class II	15.26	<0.05	26.79	<0.01	4.89	n.s.
Infilt. HLA class II	14.07	n.s.	5.36	n.s.	3.28	n.s.

HLA-DR molecule expression on tumor cells

The monoclonal antibody, EDU1 was used to stain the LC which served as a positive control. The tumor cell expression pattern with anti HLA-class II MoAb showed weak homogeneous staining in 8% of the cases

Table 5. Statistical correlation between HLA expression tumor cells and infiltrating mononuclear cells of cutaneous S.C.C.

CHARACTERISTICS	X^2	p
HLA I T v.s. HLA II T	8.28	n.s.
HLA I T v.s. HLA II L	13.78	n.s.
HLA II T v.s.HLA II L	6.78	n.s.

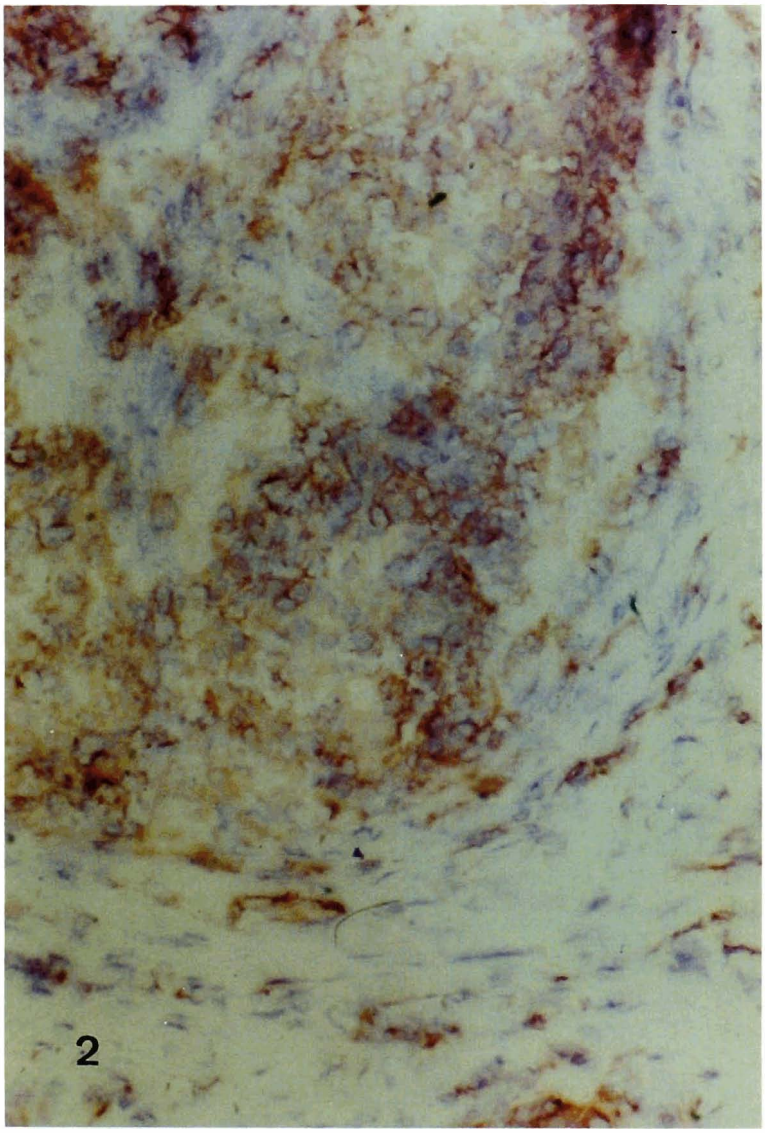

Fig. 2. Squamous cell carcinoma exhibiting uniform reactivity against HLA class II antigens. Staining EDU-1 MoAb. x 1,000

(Fig. 2), 35% scattered staining in only a few areas of the tumor, usually in peripheral areas near the mononuclear infiltrate, while the remaining 57% exhibited no significant staining for this MoAb (Fig. 3) (Table 3). The intratumoral dendritic cells found were HLA-DR positive. Class II antigen expression in tumor cells was correlated with the histopathological type and cell differentiation, whereas no correlation was observed with tumor invasion (Table 4), or between HLA class II antigen expression in tumor cells and HLA class II expression in infiltrating lymphocytes (Table 5).

CD-3 and HLA-DR molecule expression on the infiltrate

The infiltrate analyzed at this point was always peritumoral and differentiated itself from the one in the ulcerated tumors, these having an inflammatory response. In all the tumors analyzed, most infiltrating mononuclear cells were CD-3 positive and reacted to the MoAb, OKT3 (Fig. 4). Further analysis of HLA class II molecule expression in the infiltrate showed that 43% of the cases expressed class II molecules homogeneously, 13% were partially positive and 44% did not exhibit class II reactivity in these cells (Table 3). This result indicates that a large part of the peritumoral infiltrate is made up of activated T-lymphocytes. There was no correlation between HLA class II antigen expression on the infiltrate and the remaining parameters considered (Tables 4, 5).

CD1a molecule expression on tumor cells and cellular infiltrate

In the skin adjacent to the tumors, in the tumor itself and in the peritumoral infiltrate, CD1a molecule expression was studied with the monoclonal antibody, OKT6. At the level of the epidermis, OKT6-positive cells with a dendritic structure along the basal and spinous layers (Fig. 5) were found, whereas no significant differences either in distribution or in number were observed when compared to the normal skin controls which had not been exposed to the sun.

Moreover, OKT6-positive cells were always found between the peritumoral lymphocyte infiltrate and the

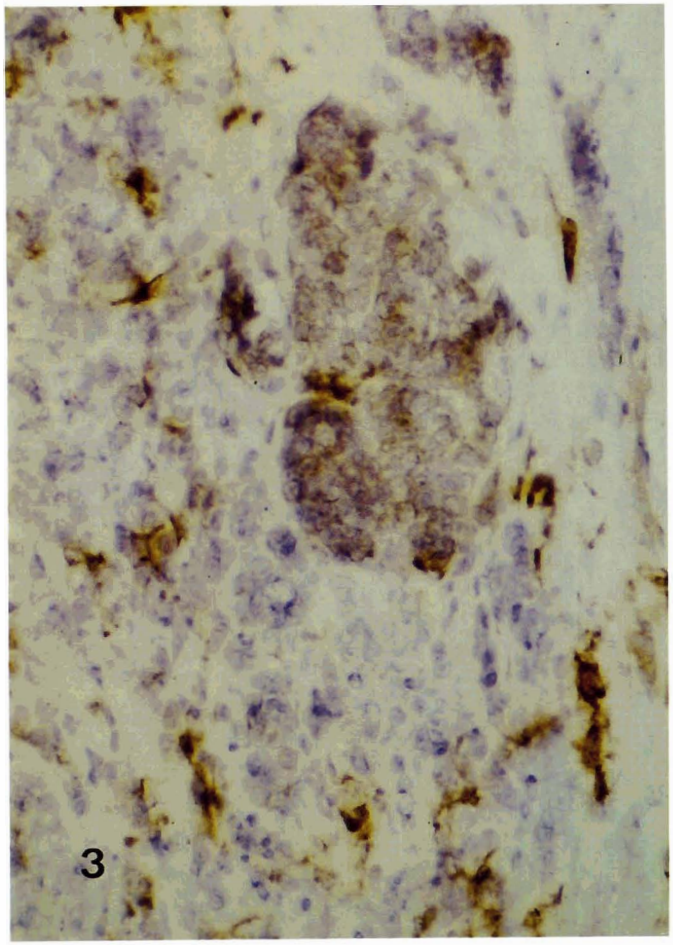

Fig. 3. Peripheral areas in squamous cell carcinoma showing HLA-DR antigen in a focal infiltrate and intratumoral dendritic cells. Staining EDU-1 MoAb. x 1,000

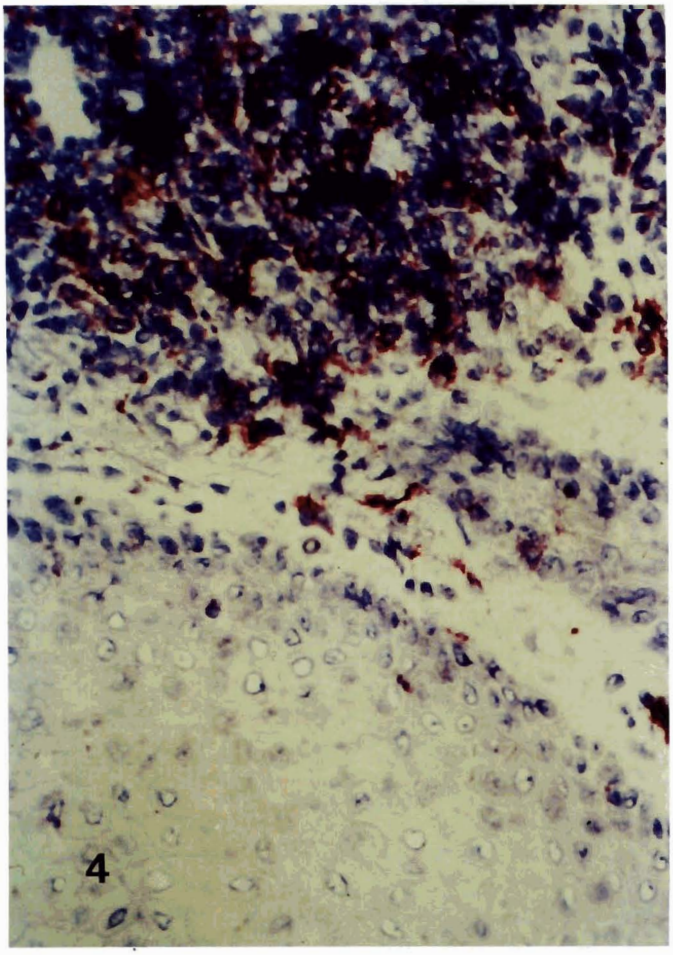

Fig. 4. Mononuclear cell infiltrate OKT3-positive adjacent to squamous cell carcinoma. Staining OKT3 MoAb. x 1,000

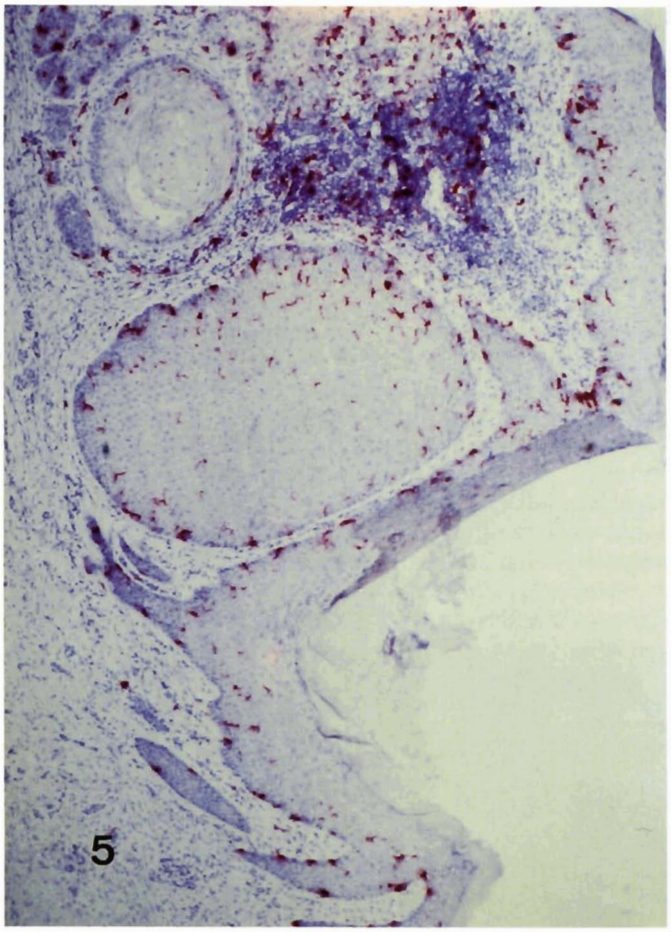

Fig. 5. OKT6 expression by Langerhans cells located in epidermis, intratumoral and in the peritumoral infiltrate. Staining OKT6 MoAb. x 200

tumor cells. A decrease in the number of intratumoral LC with respect to the number present in the epidermis adjacent to the tumor and in the control epidermis was found in 53.8% of the cases, although no morphological alterations were observed.

Discussion

A heterogeneous expression of MHC class I molecules in SCC has beeen described by several authors (Hua et al., 1985; Markey et al., 1990). Our results confirm this heterogeneous expression in different histopathological types of SCC: 30% express HLA class I molecules; 35% are partially positive; and 35% do not express these molecules. However, HLA-ABC antigen expression on tumor cells is not correlated with the histopathological type, the degree of tumor infiltration or differentiation (Table 4). The number of tumors studied in this paper lead us to question the use of HLA class I molecules as a prognosis parameters as other authors have suggested (Markey et al., 1990). On the other hand, in the studies carried out on basal cell carcinomas (BCC), our results show a correlation between the degree of cellular differentiation and HLA class I expression in tumor cells, BCC being better differentiated as these were the ones which more markedly and homogeneously expressed these antigens (García-Plata et al., 1991).

Moreover, a significant correlation is found between HLA class II antigen expression on tumor cells, the histopathological type and the degree of tumor differentiation, although this is not so with the degree of tumor invasion. This relationship indicates that most malignant or undifferentiated tumor express high levels of class II antigens. Consequently, this antigen expression can be used as a parameter for the assessment of malignancy in the.SCC (Markey et al., 1990) as has already been proposed for malignant melanoma (Bröcker et al., 1984; Ruiter et al., 1984). However, it is necessary to point out that many tumors expressed class II molecule in the peripheral areas adjacent to the cellular infiltrate, although no direct relationship between the degree of tumoral invasion has been found. Further studies completing these findings are necessary before any definite conclusions can be drawn as other authors have reached different conclusions with SCC of the larynx in which they associate class II expression and a marked cellular infiltrate with a favorable prognosis, not finding HLA class II antigen expression in the metastasis (Esteban et al., 1990). Thus, several authors have proposed that the expression or not of HLA-DR in melanic tumors should not be used to distinguish benign or malign melanocytic proliferations (Ruiter et al., 1982; West et al., 1989).

Finally, our results are not conclusive enough to state that there is a direct relationship between HLA-DR antigen expression in the tumor cells and the malignancy of the tumor. Altered expression of the major histocompatibility complex (MHC) antigens by epidermal tumours may enable them to escape the host defence mechanisms and invade surrounding tissue (Markey et al., 1990).

Our results confirm previous data that mononuclear cell infiltrates associated with these neoplasms are predominantly T-cells and LC (Hua et al., 1985; Kohchiyama et al., 1986). Moreover, 56% of these tumors have activated T-cell lymphocytes and express HLA class II antigens. Although this study does not attempt to determine the HLA-DR induction mechanisms in tumor cells, the expression, especially in the cells located near the infiltrate of the activated lymphocytes can be explained as a stimulation by gamma interference on the T-lymphocytes, as other authors have pointed out (Steeg et al., 1982; Schneeburger et al., 1986; West et al., 1989). With regard to this, Kohchiyama et al. (1986) have reported that activated T-cells are the predominant subpopulation infiltrating SCC, although they state that the presence of small numbers of NK-cells in the lesions could also contribute to the immune response against the tumor.

As to the behaviour of the LC in SCC, marked contradictions are observed in the studies carried out.

While some authors find a decreased number of cells and morphological alterations (Smolle et al., 1986; Alcalay et al., 1989), others find an increase of LC when compared to certain areas of the skin control or when the skin is adjacent to the tumor (McArdle et al., 1986). In our study with a specific LC marker (OKT6), a decrease in the number of intratumoral LC was found in 14 tumors when compared to the epidermis adjacent to the tumor and to the epidermal control. However, the morphology was always dendritic. In the remaining SCC (12 out of 26) no quantitative or qualitative variations in the cells were observed. The assessment of the number and morphology of LC can undergo noticeable variations depending on the markers used, which probably explains the marked fluctuations observed in the different studies and which Alcalay et al. (1989) already reflect in a previous study.

In summary, our results show that HLA class I and class II antigens can be differentially expressed on tumor cells from SCC. Although further studies are necessary, the analysis of HLA expression on tumor cells, especially HLA class II antigens, could contribute to set the grounds for a possible prognosis criteria.

References

Alcalay J., Goldberg L.H., Wolf J.E.Jr. and Kripke M. (1989). Variation and the number and morphology of Langerhans cells in the epidermal component of squamous cell carcinomas. Arch. Dermatol. 125, 917-920.

Bröcker E.B., Suteer L. and Sorg C. (1984). HLA-DR antigen expression in primary melanomas of the skin. J. Invest. Dermatol. 82, 244-247.

Chen H.D., Zhao Y., Sun G. and Yang G. (1988). Occurrence of Langerhans cells and expression of class II antigens on keratinocytes in malignant and benign epithelial tumors of the skin: an immunohistopathologic study with monoclonal antibodies. J. Am. Acad. Dermatol. 20, 1007-1014.

Esteban F., Ruiz-Cabello F., Concha A., Pérez-Ayala M., Sánchez-Rozas J.A. and Garrido F. (1990). HLA-DR expression is associated with excellent prognosis in squamous cell carcinoma of the larynx. Clin. Exp. Metastasis 8, 319-328.

Festenstein H., Schmith W., Alonzo A. and Leben L. (1982). Influence of selective absence of H-2Kk on Gross virus leukaemias «in vitro» and the implications for «in vivo» surveillance of leukaemia. In: Protides of the biological fluids. Peeters H. (ed). Pergamon Press. Oxford and New York. pp 231-234.

García-Espejo R., Alonso M.C., Solana R., Pera C. and Peña J. (1986). Expression of HLA molecules of cells from fresh explants of human digestive tract cancer. J. Immunogenet. 13, 211-215.

García-Plata M.D., Mozos E., Sierra M.A., Peña J. and Solana R. (1991). HLA expression in basal cell carcinomas. Invasion Metastasis 11, 166-173.

Habets J.M., Tank B. and van Joost T. (1989). Characterisation of the mononuclear infiltrate in Bowen's disease squamous cell carcinoma in situ). Evidence for a the cell-mediated anti-tumor immune response. Virchows Arch. (A) 415, 125-130.

Hirschberg H., Braathen L.R. and Thorsby E. (1982). Antigens presentation by vascular, endothelial and epidermal Langerhans cells, the role of HLA-DR. Immunol. Rev. 66, 57-77.

Hua L.A., Kagen C.N., Carpenter R.J. and Goltz A.R. (1985). HLA and beta 2-microglobulin expression in basal and squamous cell carcinomas of the skin. Int. J. Dermatol. 24, 660-663.

Hui K., Grosveld F. and Festenstein H. (1984). Rejection of transplantable AKR leukaemia cells following MHC DNA-mediated cell transformation. Nature 311, 750-751.

Kohchiyama A., Oka D. and Ueki H. (1986). Immunohistologic studies of squamous cell carcinoma: Possible participation of leu-7+ (Natural killer) cells as antitumor effector cells. J. Invest. Dermatol. 87, 515-518.

Markey A.C., Churchill L.J. and McDonald D.M. (1990). Altered expression of major histocompatibility complex (MHC) antigens by epidermal tumours. J. Cutan. Pathol. 17, 65-71.

McArdle J.P., Knight B.A., Halliday G.M., Muller H.K. and Rowden G. (1986). Quantitative assessment of Langerhans cells in actinc keratosis, Bowen's disease, keratoacanthoma, squamous cell carcinoma and basal cell carcinoma. Pathology 18, 212-216.

Momburg F., Möller P., Moldenhauer G. and Hammerling G.H. (1986). Loss of HLA-A,B,C in colorectal carcinoma is related to the degree of differentiation. J. Immunogenet. 13, 195-199.

Momburg F., Ziegler A., Harpprecht J., Möller P., Moldenhauer G. and Hammerling G.H. (1989). Selective loss of HLA-A or HLA-B antigen expression in colon carcinoma. J. Immunol. 142, 352-355.

Murphy G.F., Bhan F., Sato S., Mihm M.C. Jr. and Harris T.J. (1981). A new immunologic marker for human L-cells. New Eng. J. Med. 304, 791-795.

Murphy G.F., Ershler W.B., Krusinski P.A. and Albertini R.J. (1982). Evaluation of local and systemic immunocomponent cells in patients with multiple skin cancers (MSC). Clin. Res. 30, 599-603.

Natali P.G., Cordialy-Fei P., Cavaliere R., Di Filippo F., Quaranto V., Pellegrino M.A. and Ferrone S. (1981). Ia-like antigens on freshly explanted human melanoma. Clin. Immunol. Immunopathol. 19, 250-255.

Natali P.G., Viora M., Nicotra M.R., Cordiali-Fei P., Cavaliere R. and Ferrone S. (1983). Antigenic heterogeneity of skin tumors of nonmelanocyte origin: Analysis with monoclonal antibodies to tumor-associated antigens and to histocompatibility antigens. JNCI. 71, 439-447.

Natali P.G., Bigotti A., Cavaliere R., Nicotra M.R. and Ferrone S. (1984). Phenotyping of lesions of melanocyte origin with monoclonal antibodies to melanoma-associated antigens and to HLA antigens. J. Natl. Cancer Inst. 73, 13-24.

Niedecken H., Lutz G., Bauer R. and Kreysel H.W. (1988). Differential expression of major histocompatibility complex class II antigens on human keratinocytes. J. Am. Acad. Dermatol. 19, 1030-1037.

Quigstad E. and Thorsby E. (1983). Class-HLA restriction of antigen-specific human T-lymphocyte clones. Scand. J. Immunol. 18, 299-306.

Ruiter D.J., Bhan A.K., Harrist T.J., Sober A.J. and Mihm M.C. (1982). Major histocompatibility antigens and mononuclear inflammatory infiltrate in benign nevomelanocytic proliferations and malignant melanoma. J. Immunol. 129, 2808-2814.

Ruiter D.J., Bergman W., Welvaart K., Scheffer E., Van Vloten W.A., Russo C. and Ferrone S. (1984). Immunohistochemical analysis of malignant melanomas and nevocellular nevi with monoclonal antibodies to distinct monomorphic determinants of HLA-antigens. Cancer Res. 44, 3930-3935.

Schmith W. and Festenstein H. (1982). Resistance to cell-mediated cytotoxicity is correlated with reduction of H-2K gene products in

AKR leukaemia. Immunogenetics 16, 257-261.

Schneeburger E.E., De Ferrari M., Skoskiewcz M.J., Russell P.S. and Kolvi R.B. (1986). Induction of MHC-determined antigens in the lung by interferon-gamma. Lab. Invest. 55, 138-144.

Smolle J., Soyer H.P., Ehall R., Bartenstein S. and Kerl H. (1986). Langerhans cell density in epithelial skin tumors correlated with epithelial differentiation but not with the peritumoral infiltrate. J. Invest. Dermatol. 87, 477-479.

Steeg P.S., Moore R.N., Johnson H.M. and Openheim J.J. (1982). Regulation of murine macrophage Ia antigen expression by a lymphokine with immune interferon activity. J. Exp. Med. 156, 1780-1793.

Tanaka K., Isselbacher K.J., Khoury G. and Jay G. (1985). Reversal of oncogenesis by the expression on an MHC class I gene. Science 228, 26-31.

Vánky F., Roberts T., Klein E. and Willems J. (1987). Auto-tumor immunity in patients with solid tumors: Participation of CD3 complex and MHC class I antigens in the lytic interactions. Immunol. Lett. 16, 21-26.

Vánky F., Stuber G., Willems J., Sjowall K., Larsson B., Book K., Ivert T., Peterffy A. and Klein E. (1988). Importance of MHC antigen expression on solid tumors in the in vitro interactions with autologous blood lymphocytes. Cancer Immunol. Immunother. 27, 213-219.

Vánky F., Stuber G., Rotsein S. and Klein E. (1989). Auto-tumor recognition following in vitro induction of MHC antigen expression on solid human tumors: stimulation of lymphocytes and generation of cytotoxicity against the original MHC antigen negative tumor cells. Cancer Immunol. Immunother. 28, 17-22.

Wallich R., Bulbuc N., Hammerling G.J., Katzau S., Feldman M. and Segal S. (1985). Abrogation of metastatic properties of tumor cells by the novo expression of H-2K antigens following H-2 gene transfection. Nature 315, 301-305.

West H.P., Priyakumar P., Jagjivan R. and Colldoy P.S. (1989). Can HLA-DR expression help in the routine diagnosis of malignant melanomas? Br. J. Dermatol. 121, 175-178.

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