

Local immune response in serous papillary carcinoma of the endometrium

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Summary. Objective: Serous papillary carcinomas of the endometrium are aggressive tumors that tend to permeate, in a very extensive fashion, to uterine and adnexal lymphatic and vascular channels at an early stage in their evolution, and are associated with a particularly gloomy prognosis. It is generally thought that even tumors apparently limited to the endometrium or confined to an endometrial polyp have a poor outcome. Our study points towards the value of HLA-DR antigen in the outcome of serous papillary endometrial cancer. Our aim was to assess the HLA-DR expression in inactive, endometrial intraepithelial carcinoma (EIC), and invasive serous carcinoma curettage specimens from the endometrial cavity, suggesting a role in immune response to keep tumor proliferation in check. Study design: Thirty-one cases of inactive endometrium, twelve cases of EIC, and thirty-nine cases of serous papillary invasive carcinoma curettages were evaluated for the detection of HLA-DR monoclonal antigen. T helper (T_H) marker (CD4) in the tumor stroma of the relevant cases was also studied, given that it is now known that the dependence of immune responsiveness on the class II antigens reflects the central role of these molecules in presenting antigen to T_H cells. Results: HLA-DR was expressed in 20 of 31 inactive endometrium (64.5%), 4 of 12 in EIC (33.3%), and in 10 of 39 serous papillary invasive carcinomas (25.6%). CD4 was expressed in 9 of 31 inactive endometrium (29%), 5 of 12 in EIC (42%), and in 26 of 39 serous papillary invasive carcinomas (67%). Conclusions: The results showed decreased expression of HLA-DR and increased expression of CD4 as the lesion progressed to malignancy. The aberrant expression of HLA-DR by epithelial cells of inactive endometrium, of EIC and of serous papillary invasive

carcinomas agrees with the hypothesis of the inactive endometrium - carcinoma in situ sequence as the usual route for the development of serous papillary invasive carcinoma. The immune attract mechanism by low HLA-DR signaling seems to be of minor importance in the malignant and metastatic potential of the serous papillary endometrial tumours.

Key words: HLA-DR, C4, Endometrium, Carcinoma

Introduction

The major histocompatibility complex is a series of genes that participate in the regulation of the immune response. This complex encodes two classes of cell-surface glycoprotein antigens: class I, found in all nucleated cells; and class II antigens, normally found only in a limited number of cells (B lymphocytes, macrophages, Langerhans' cells, dendritic cells, vascular endothelial cells and some epithelial cells) (Daar et al., 1984; Lafuse and David, 1984). Class II antigens control cellular interactions between lymphocytes. In man at least three class II antigens (DR, DQ, and DP), each consisting of a and β glycoproteins chains, are encoded by the HLA-D region of chromosome 6 (Klein, et al., 1983; Steinmetz and Hood, 1983; Lafuse and David, 1984).

The majority of pathogens gain access to the body at a mucosal site, and the epithelial cells lining the lumen of the mucosa provide the first barrier against their invasion. In addition to their barrier, absorption and transport functions, epithelial cells play an important role in both innate and adaptive immune responses. They secrete soluble molecules including defensins (Eisenhauer et al., 1992; O'Neil et al., 1999) and complement components (Andoh et al., 1993) that neutralize and inactivate microorganisms and their toxins. In addition, they can present foreign antigens to T cells affecting their proliferation, cytolytic activity and

cytokine production. Typically, antigen-presenting cells are bone marrow derived cells such as dendritic cells, macrophages (MO) and monocytes (i.e. professional antigen presenting cells). However, certain other cell types including intestinal epithelial cells (Bland and Warren, 1986; Andoh et al., 1993; Hershberg et al., 1997), renal tubular epithelial cells (Kelly and Singer, 1993), keratinocytes (Nickoloff and Turka, 1994) and endothelial cells (Savage et al., 1995) have been shown to function in a limited context as antigen presenting cells, which are characteristically less efficient at antigen processing and presentation and are thus referred to as non-professional antigen presenting cells.

Epithelial cells can transport antigens from the lumen by a process of transcytosis for eventual processing and presentation by professional antigen presenting cells found in the underlying sub-epithelial stroma. The transcellular transport of antigen by epithelial cells is generally a slow process but may be enhanced by immunization (Berin et al., 1997). Studies by Blumberg and co-workers have demonstrated functional MHC class I related IgG receptor (FcRn) on intestinal epithelial cells (Israel et al., 1997; Dickinson et al., 1999). Since both the female reproductive tract (FRT) and the gut have IgG, which increases in disease states, FcRn may facilitate transport of IgG-antigen complexes through epithelial cells into the basolateral sub-epithelium where antigen presenting cells and T cells reside. Previous studies have reported the presence of antigen presenting cells including macrophages (MO) and B cells throughout the FRT (Givan et al., 1997). Lymphoid aggregates consisting of a central core of B cells surrounded by numerous T cells, which are in turn circumscribed by MO, are shown (Yeaman et al., 1997). Other antigen presenting cells including dendritic cells have been observed in the lower tract although their presence in the upper tract remains controversial (Poppe et al., 1998).

Recent studies have established that intestinal epithelial cells can express MHC class II molecules and present antigen directly to CD4+ T cells (Kaiserlian et al., 1989; Hershberg et al., 1997; Kaiserlian, 1999).

We report that there is a loss in the HLA-DR expression by the epithelial cells and a gain in the CD4 expression in the endometrial stroma, during the progression from inactive endometrium to invasive serous papillary carcinoma.

Materials and methods

A review was made of 31 consecutive cases of inactive endometrium, 12 cases of carcinoma in situ, and 39 cases of invasive serous carcinoma from January 1999 to December 2003. For all patients, diagnostic curettage and hysterectomy specimens, intraoperative frozen sections and paraffin sections were available. The specimens were obtained from postmenopausal females aged 55-78 years. Representative 4 µm sections from formalin fixed, paraffin embedded curettage specimens

were histologically classified as inactive endometrium, carcinoma in situ and invasive serous carcinoma types. All carcinomas in situ were grade 1, and all invasive serous tumors were nuclear grade 3, by definition. Further sections from the same tissue were submitted to immunohistochemical reactions for HLA-DR, and CD4 monoclonal antigens (DAKO). Differential diagnosis of carcinoma in situ from invasive carcinoma curettages was based on the criteria by Kurman and Norris (1982): desmoplastic stromal response, cribriform pattern occupying half of a low power field, complex papillary pattern occupying half of an original magnification; all three features were indicative of stromal invasion. Hysterectomy was performed 1 month after curettage. In accordance to the disease stage, the histological parameters evaluated were depth of myometrial invasion, extent in the cervix and lymph node involvement. The Regional Ethics Committee approved the study. Written informed consent was obtained from all patients and the procedures were in accordance with the institutional guidelines.

Immunohistochemistry

Immunohistochemistry was performed with the various antibodies used on serial sections. Tissue sections (4 µm) were deparaffinized, rehydrated, and treated with 0.3 per cent hydrogen peroxide for 5 min to quench endogenous peroxidase activity. Non-specific binding was blocked with serum for 10 min. Slides were then incubated for 30 min with the monoclonal antibodies (1/40), namely mouse anti-human HLA-DR, Alpha-Chain (DAKO) and CD4 (DAKO). Control slides were incubated for the same period with normal mouse serum. After several 10 min washes in PBS, samples were developed with the peroxidase LSAB kit (labelled streptavidin - biotin method, DAKO), which allows the detection of the first antibody. The slides were briefly counterstained with Mayer's haematoxylin, mounted, and examined under an Olympus BX40 microscope.

The immunostained sections were examined with a x40 objective and the distribution of HLA-DR and CD4 within the cell was recorded. Every stained cell was scored as positive regardless of staining intensity. To count the number of cells with HLA-DR and CD4 stainings, a 10x10 square calibrated grid was inserted into the eyepiece of an Olympus binocular microscope.

Five-to-ten fields were examined for each section, and at least 1000 cells were scored, depending on cellularity. The percentage of positive cells was recorded as the HLA-DR and CD4 indices. The statistical analysis was obtained by the t-test. The mean values were expressed as average ± SD.

$$\text{HLA DR index} = \frac{\text{no. of positive cells}}{\text{no. total (positive + negative cells)}}$$

$$\text{CD4 index} = \frac{\text{no. of positive cells}}{\text{no. total (positive + negative cells)}}$$

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The indices ranged from 0-100%, with a mean of 18%. The mean index was evaluated in three ranges: low index (under 18%), grade I; moderate index (from 18 to 50%), grade II; and high index (from 51 to 100%), grade III.

Results

The sections were examined independently by two

observers, and positive cellular staining for HLA-DR and CD4 antigens were manifested as fine red cytoplasmic expression (Figs. 1-5). Our findings are summarized in Tables 1-3).

HLA-DR was expressed in 20 of 31 of inactive endometrium (64.5%) (48.61 ± 1.43 cells/mm²), in 4 of 12 EIC (33.3%) (32.72 ± 1.91 cells/mm²), and in 10 of 39 invasive serous papillary carcinomas (25.6%) (11.37 ± 3.29 cells/mm²). Of 20 positive inactive

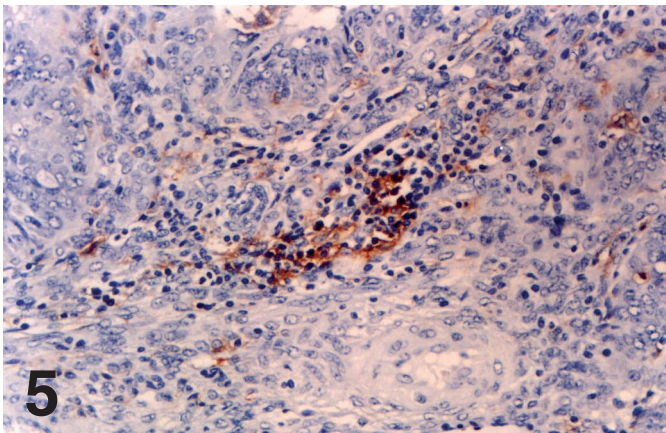
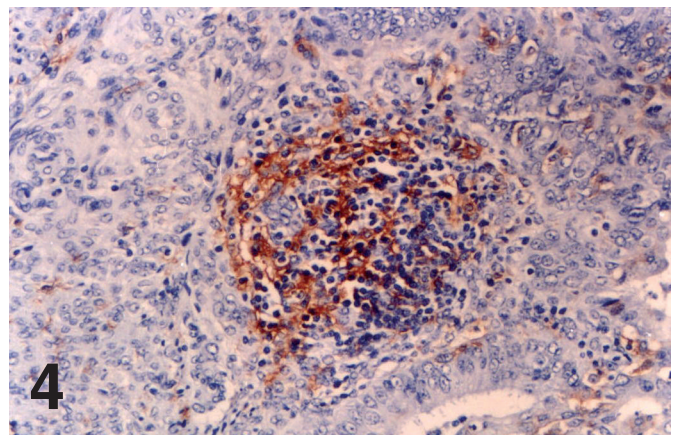
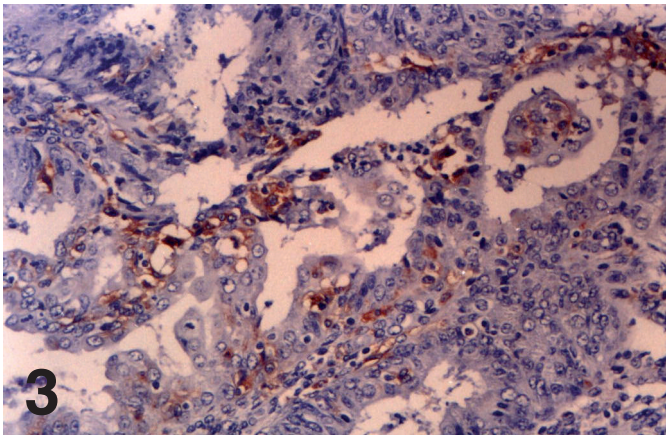
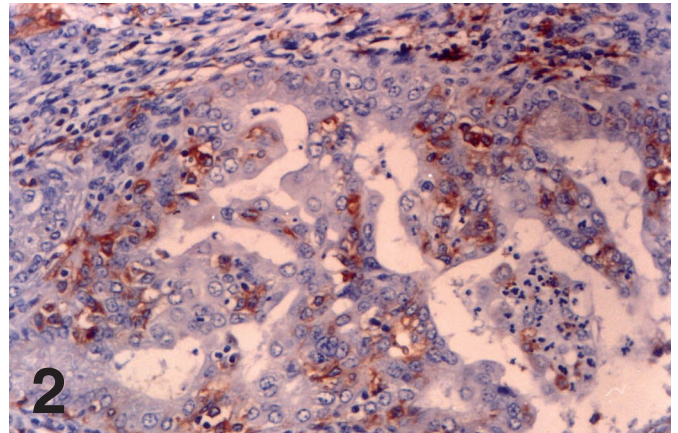
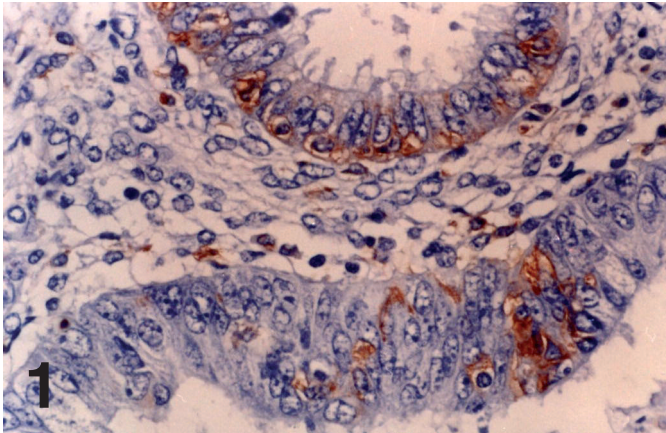


Fig. 1. Inactive endometrium: HLA-DR expression. Immunostain, x 200

Fig. 2. EIC: HLA-DR expression. Immunostain, x 200

Fig. 3. Serous papillary carcinoma with stromal lymphocytic infiltrate: HLA-DR expression. Immunostain, x 400

Fig. 4. Inactive endometrium: Stromal CD4 expression. Immunostain, x 200

Fig. 5. Serous papillary carcinoma: Stromal CD4 expression. Immunostain, x 200

endometrium 9 were scored as HLA-DR grade II and 11 as HLA-DR grade III. Of 4 positive EIC 1 was scored as HLA-DR grade I, and 3 as HLA-DR grade II. Of 10 positive invasive serous papillary carcinomas 1 was scored as HLA-DR grade I, 7 as grade II, and 2 as grade III.

CD4 was expressed in 9 of 31 inactive endometrium (29%) (55.41±3.12 cells/mm²), in 5 of 12 carcinomas in situ (42%) (67.93±4.35 cells/mm²), and in 26 of 39 invasive serous papillary carcinomas (67%) (101.46±6.76 cells/mm²). Of 9 positive inactive endometrium 4 were scored as CD4 grade II and 5 as CD4 grade III. Of 5 positive EIC 1 was scored as CD4 grade I, 3 as CD4 grade II and 1 as CD4 grade III. Of 26 positive invasive serous papillary carcinomas 4 were scored as CD4 grade I, 12 as CD4 grade II, and 10 as CD4 grade III.

Our results demonstrated a statistically significant difference in inactive endometria concerning the HLA-DR expression over the equivalent expression of the antigen in carcinomas in situ and invasive serous tumors (p<0,0001 respectively). Similar changes were found concerning the CD4 expression in inactive endometria over carcinomas in situ and invasive serous carcinomas. These data suggest that HLA-DR was mediated by stromal T-helper cells as CD4 positive infiltrates were observed in all biopsies examined.

No correlation of our data with staging of the disease in the hysterectomy specimens could be made as our cases of invasive serous tumors were all stage FIGO II (all cases showed cervical glandular or stromal

involvement). Also no correlation with myometrial invasion (in all cases subserosal), lymph node status (all cases negative), lymphovascular space involvement (all cases negative), was made.

Discussion

Major histocompatibility complex antigens (MHC), or human leukocyte antigens (HLA) in humans, are considered to be essential when tumor cells are recognized and attacked by host immune cells. Therefore, the tumor growth may be affected by the states of HLA expression. In various neoplasms, the grade of HLA expression has been clinically reported to be associated with the degree of differentiation and the prognosis regarding both class I (van Duinen et al., 1988; Dammrich et al., 1990; Goepel et al., 1991; Pantel et al., 1991; Redondo et al., 1991; Scupoli et al., 1996) and class II antigens (van Duinen et al., 1988; Esteban et al., 1989; Brunner et al., 1991; Redondo et al., 1991; Scupoli et al., 1996). However, contradictory results have been also reported (Ghosh et al., 1986; Natali et al., 1986; Stein et al., 1988; Connor and Stern, 1990; Wintzer et al., 1990). Such controversy is probably not only due to the different tissue origins of various tumors but also due to the heterogeneous expression of individual tumor cells. It is difficult to quantitatively evaluate the heterogeneity of HLA expression using conventional tissue sections for a histologic examination. The dispersed cells of fresh tumor tissues most likely represent the whole population of tumor cells and are thus advantageous to the quantitative assessment of HLA expression.

To elucidate the clinico-biological significance of HLA expressed on neoplastic cells, we have quantitatively assessed the degrees of the class II expression using paraffin-embedded neoplastic cells, and also the grade of T helper lymphocytic infiltration in inactive endometrium, carcinoma in situ, and serous papillary carcinoma of the endometrium.

In the present study, we clearly demonstrated a loss of HLA-DR expression from inactive endometrium

Table 1. Extent of HLA-DR and CD4 immunoreactivity in inactive, EIC, and invasive serous carcinoma of the endometrium (percentage of reactive cells).

	EXTENT OF HLA-DR IMMUNOREACTIVITY			EXTENT OF CD4 IMMUNOREACTIVITY		
	1	2	3	1	2	3
Diagnosis						
Inactive	-	9	11	-	4	5
EIC	1	3	-	1	3	1
Invasive Ca	1	7	2	4	12	10

Table 2. Cytoplasmic intensity of HLA-DR and CD4 immunoreactivity in inactive, EIC and invasive serous carcinoma of the endometrium (0, no staining; +, weak cytoplasmic staining; ++, moderate cytoplasmic staining; +++, marked cytoplasmic staining).

	CYTOPLASMIC INTENSITY OF HLA-DR IMMUNOREACTIVITY				CYTOPLASMIC INTENSITY OF CD4 IMMUNOREACTIVITY			
	0	+	++	+++	0	+	++	+++
DIAGNOSIS								
Inative	-	4	12	4	-	1	6	2
EIC	1	3	-	-	-	1	4	-
Invasive Ca	1	-	5	4	1	3	15	7

Table 3. Expression of HLA-DR and CD4 in endometrial curettage specimens.

	INACTIVE	EIC	SA*
	HLA-DR (+) CELLS/MM2	48.61±1.43	32.72±1.91
CD4 (+) CELLS/MM2	55.41±3.12	67.93±4.35	p<0.0001
	EIC	INVASIVE SEROUS Ca	SA*
	HLA-DR (+) CELLS/MM2	32.72±1,91	11.37±3.29
CD4 (+) CELLS/MM2	67.93±4.35	101.46±6.76	p<0.0001

SA*: statistical analysis

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towards serous papillary carcinoma. It is well known that HLA class II antigens are usually expressed on such immune cells as macrophages, B cells and activated T cells and that they are also involved in antigen presentation as well as in the regulation of the helper T cell function. A number of studies have also revealed the expression of class II antigens by both various non-immune normal and malignant cells (Ghosh et al., 1986; Natali et al., 1986; van Duinen et al., 1988; Esteban et al., 1989; Wintzer et al., 1990; Brunner et al., 1991; Redondo et al., 1991), although the biological significance of the class II expression of such cells remains unclear.

On the other hand, in view of immunological aspects, the class II expression of tumor cells has been reported to correlate with the local infiltration of lymphocytes (Dammrich et al., 1991; Kamma et al., 1991). In the present study, expression of HLA-DR by epithelial neoplastic cells was possibly mediated by stromal T helper lymphocytes as lymphoid cell infiltrates were observed in all biopsy specimens containing HLA-DR positive neoplastic cells. The increased aberrant expression of HLA-DR in tumor cells has been viewed as an important feature to escape tumor recognition by immune cells, and correlates with high-grade malignancy and enhanced metastatic potential. We have demonstrated progressive downregulation of HLA-DR antigen expression from inactive, EIC and invasive serous carcinoma, in our series of endometria. Downregulation of the expression of particular class I loci or loss of genes for particular class I-alpha chains is another escape mechanism, and yet another is downregulation of certain proteasome component molecules as exhibited by small cell lung carcinoma (Othieno-Abinya, 2003). These concepts can be employed in approach to anticancer treatment. Observations that renal cell carcinoma and malignant melanoma can undergo spontaneous regression have strengthened the belief that enhanced immune system is capable of eradicating established tumor cells (Othieno-Abinya, 2003). With recent advances in immunology, the biologic basis for antitumor immunity is beginning to unfold. T lymphocytes can respond to tumor antigens presented as peptides in association with MHC molecules and tumor cells or on "professional" antigen presenting cells such as dendritic cells, mounting on immune response to keep tumorigenesis in control.

In our series of endometria, there was a decreased expression of HLA-DR from inactive to malignant process and a subsequent increased immune response, providing new insights for a better understanding of the tumorhost relationships in this severe form of neoplasia.

References

Andoh A., Fujiyama Y., Bamba T. and Hosoda S. (1993). Differential cytokine regulation of complement C3, C4, and factor B synthesis in human intestinal epithelial cell line, Caco-2. *J. Immunol.* 51, 4239-4247.

- Berin M.C., Kiliaan A.J., Yang P.C., Groot J.A., Taminiau J.A. and Perdue M.H. (1997). Rapid transepithelial antigen transport in rat jejunum: impact of sensitization and the hypersensitivity reaction. *Gastroenterology* 113, 856-864.
- Bland P.W. and Warren L.G. (1986). Antigen presentation by epithelial cells of the rat small intestine. I. Kinetics, antigen specificity and blocking by anti-Ia antisera. *Immunology* 58, 1-7.
- Brunner C.A., Gokel, J.M., Riethmullerand G., and Johnson J.P. (1991). Expression of HLA-D subloci DR and DQ by breast carcinomas is correlated with distinct parameters of favourable prognosis. *Eur. J. Cancer* 27, 411-416.
- Connor M.E., and Stern P.L. (1990). Loss of MHC class-I expression in cervical carcinomas. *Int. J. Cancer* 46, 1029-1034.
- Daar A.S., Fuggle S.V., Fabre J.W., Ting A. and Morris P.J. (1984). The detailed distribution of MHC class II antigens in normal human organs. *Transplantation* 38, 293-298.
- Dammrich J., Muller-Hermelink H.K., Mattner A., Buchwald J., and Ziffer S. (1990). Histocompatibility antigen expression in pulmonary carcinomas as indication of differentiation and of special subtypes. *Cancer* 65, 1942-1954.
- Dammrich J., Buchwald J., Papadopoulos T. and Muller-Hermelink H.K. (1991). Special subtypes of pulmonary adenocarcinomas indicated by different tumor cell HLA-expression and stromal infiltrates. *Virchows Arch (B)* 61, 9-18.
- Dickinson B.L., Badizadegan K., Wu Z., Ahouse J.C., Zhu X., Simister N.E., Blumberg R.S. and Lencer W.I. (1999). Bidirectional FcRn-dependent IgG transport in a polarized human intestinal epithelial cell line. *J. Clin. Invest.* 104, 903-911.
- Eisenhauer P.B., Harwig S.S. and Lehrer R.I. (1992). Cryptdins: antimicrobial defensins of the murine small intestine. *Infect. Immun.* 60, 3556-3565.
- Esteban F., Concha, A., Huelin, C., Perez-Ayala M., Pedrinaci S., and Ruiz-Caballo F. (1989). Histocompatibility antigens in primary and metastatic squamous cell carcinoma of the larynx. *Int. J. Cancer* 43, 436-442.
- Givan A.L., White H.D., Stern J.E., Colby E., Gosselin E.J., Guyre P.M. and Wira C.R. (1997). Flow cytometric analysis of leukocytes in the human female reproductive tract: comparison of fallopian tube, uterus, cervix, and vagina. *Am. J. Reprod. Immunol.* 38, 350-359.
- Ghosh A.K., Moore M., Street A.J., Howat J.M.T. and Schofield P.F. (1986). Expression of HLA-D sub-region products on human colorectal carcinoma. *Int. J. Cancer* 38, 459-464.
- Goepel J.R., Rees R.C., Rogers K., Stoddard C.J., Thomas W.E.G. and Shepherd L. (1991). Loss of monomorphic and polymorphic HLA antigens in metastatic breast and colon carcinoma. *Br. J. Cancer* 64, 880-883.
- Hershberg R.M., Framson P.E., Cho D.H., Lee L.Y., Kovats S., Beitz J., Blum J.S. and Nepom G.T. (1997). Intestinal epithelial cells use two distinct pathways for HLA class II antigen processing. *J. Clin. Invest.* 100, 204-215.
- Israel E.J., Taylor S., Wu Z., Mizoguchi E., Blumberg R.S., Bhan A. and Simister N.E. (1997). Expression of the neonatal Fc receptor, FcRn, on human intestinal epithelial cells. *Immunology* 92, 69-74.
- Kaiserlian D. (1999). Epithelial cells in antigen. Sampling and presentation in mucosal tissues. *Curr. Top. Microbiol. Immunol.* 236, 55-78.
- Kaiserlian D., Vidal K. and Revillard J.P. (1989). Murine enterocytes can present soluble antigen to specific class II-restricted CD4+ T cells. *Eur. J. Immunol.* 19, 1513-1516.

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- Kamma H., Yazawa T., Ogata T., Horiguchi H., and Iijima T. (1991). Expression of MHC class II antigens in human lung cancer cells. *Virchows Arch. (B)* 60, 407-412.
- Kelley V.R. and Singer G.G. (1993). The antigen presentation function of renal tubular epithelial cells. *Exp. Nephrol.* 1, 102-111.
- Klein J., Figuerou F. and Nagy A. (1983). Genetics of the major histocompatibility complex: the final act. *Annu.Rev. Immunol.* 1, 119-142.
- Kurman R.J. and Norris H.J. (1982). Evaluation of criteria for distinguishing atypical endometrial hyperplasia from well-differentiated carcinoma. *Cancer* 49, 2547-2559.
- Lafuse W.P. and David C.S. (1984). Ia antigens. Genes molecules, and function. *Transplantation* 38, 443-453.
- Natali P., Bigotti A., Cavalieri R., Nicotra M.R., Tecce R., Manfredi D., Chen Y.X., Nadler L.M. and Ferrone S. (1986). Gene products of the HLA-D region in normal and malignant tissues of nonlymphoid origin. *Hum. Immunol.* 15, 220-233.
- Nickoloff B.J. and Turka L.A. (1994). Immunological functions of non-professional antigen-presenting cells: new insights from studies of T-cell interactions with keratinocytes. *Immunol. Today* 15, 464-469.
- O'Neil D.A., Porter E.M., Elewaut D., Anderson G.M., Eckmann L., Ganz T. and Kagnoff M.F. (1999). Expression and regulation of the human beta-defensins hBD-1 and hBD-2 in intestinal epithelium. *J. Immunol.* 163, 6718-6724.
- Othieno-Abinya NA, (2003). HLA system and cancer. *East Afr. Med. J.* 80, 553 (Editorial).
- Pantel K., Schlimok G., Kutter D., Schaller G., Genz T., Wiebecke B., Backmann R., Funke I. and Riethmuller G. (1991). Frequent down-regulation of major histocompatibility class I antigen expression on individual micrometastatic carcinoma cells. *Cancer Res.* 51, 4712-4715.
- Poppe W.A., Drijkoningen M., Ide P.S., Lauweryns J.M. and Van Assche F.A. (1998). Lymphocytes and dendritic cells in the normal uterine cervix. An immunohistochemical study. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 81, 277-282.
- Redondo M., Concha A., Oldiviola R., Cueto A., Gonzalez A., Garrido F. and Ruiz-Cabello F. (1991). Expression of HLA class I and II antigens in bronchogenic carcinomas: its relationship to cellular DNA content and clinical-pathological parameters. *Cancer Res.* 51, 4948-4954.
- Savage C.O., Brooks C.J., Harcourt G.C., Picard J.K., King W., Sansom D.M. and Willcox N. (1995). Human vascular endothelial cells process and present autoantigen to human T cell lines. *Int. Immunol.* 7, 471-479.
- Scupoli M.T., Sartoris S., Tosi G., Ennas M.G., Nicolis M., Cestari T., Zamboni G., Martignoni G., Lemoine N.R., Scarpa A., and Accola R.S. (1996). Expression of MHC class I and class II antigens in pancreatic adenocarcinomas. *Tissue Antigens* 48, 301-311.
- Stein B., Momburg F., Schwarz V., Schlag P., Moldenhauer G., and Muller P. (1988). Reduction or loss of HLA-A, B, C antigens in colorectal carcinoma appears not to influence survival. *Br. J. Cancer* 57, 364-368.
- Steinmetz M. and Hood L. (1983). Genes of the major histocompatibility complex in mouse and man. *Science* 222, 727-733.
- van Duinen S.G., Ruiters D.J., Broecker E.B., van der Velde E.A, Sorg C., Welnaart K., and Ferrone S. (1988). Level of HLA antigens in locoregional metastases and clinical course of the disease in patients with melanoma. *Cancer Res.* 48, 1019-1025.
- Wintzer H.O., Benzing M. and von Kleist S. (1990). Lacking prognostic significance of 2-microglobulin, MHC class I and class II antigen expression in breast carcinomas. *Br. J. Cancer* 62, 289-295
- Yeaman G.R., Guyre P.M., Fanger M.W., Collins J.E., White H.D., Rathbun W., Orndorff K.A., Gonzalez J., Stern J.E. and Wira C.R. (1997). Unique CD8+ T cell-rich lymphoid aggregates in human uterine endometrium. *J. Leukoc. Biol.* 61, 427-435.

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