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Expression of Major Histocompatibility Class I and Class II Antigens and Intercellular Adhesion Molecule-1 on Operable Non-small Cell Lung Carcinomas: Frequency and Prognostic Significance

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Major histocompatibility complex (MHC) antigens and adhesion molecules, such as the intercellular adhesion molecule-1 (ICAM-1), appear to play an important role in the immunological recognition and destruction of tumour cells. We, therefore, examined the expression patterns of these proteins on primary tumours of 91 patients with operable non-small cell lung cancer (NSCLC). Applying immunohistochemistry with monoclonal antibody (MAb) W6/32 against a common framework determinant of HLA class I antigens revealed a deficient expression in 33.0% of the cases analysed, while neo-expression of either HLA class II antigens (MAb TAL.1B5) or ICAM-1 (MAb PA3.58-14) was observed in 26.4 or 29.7% of tumours, respectively. Analysis of consecutive tumour specimens indicated that HLA antigens and ICAM-1 were frequently coexpressed. With regard to clinicopathological risk factors, we could demonstrate a preferential expression of those markers in patients with locally restricted and well-differentiated tumours or no lymph node metastases, which was more pronounced in adenocarcinomas than in squamous cell carcinomas. In contrast, the presence versus the absence of HLA antigens and ICAM-1 was not correlated with the rate of tumour recurrence or overall survival in patients with NSCLC. In conclusion, the co-ordinated expression of immunologically relevant cell surface molecules on primary NSCLC is a frequent event that correlates with distinct parameters of favourable prognosis. However, we have no evidence that the immune response facilitated by these molecules can effectively influence the clinical course of the disease.

Key words: NSCLC, MHC molecules, adhesion molecules, prognosis
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INTRODUCTION

LUNG CANCER is currently the leading cause of cancer-related death [1] in western industrial nations with an overall 5-year survival rate of approximately 10% [2]. Most patients are inoperable at the time of diagnosis, and metastatic relapse in patients with operable non-small cell lung carcinomas (NSCLC) remains a frequent event [3].

HLA molecules and the intercellular adhesion molecule-1 (ICAM-1) play an important role in the human immune response, involving T cell recognition and other lymphocyte effector functions. HLA class I molecules are expressed on most nucleated cells, and act as restriction elements for the recognition of foreign antigens by T cells. In contrast, HLA class II molecules are constitutionally expressed primarily on B cells, while being inducible on many other cell types by a variety of immunoregulatory molecules. There has been evidence that the loss of HLA class I expression and the acquisition HLA class II expression occurs frequently on primary and metastatic tumour cells [4–7] and that this might have a prognostic significance in some tumours, such as breast cancer and melanoma [6, 8]. Although downregulation of HLA class I expression occurs on primary NSCLC [9,10], its prognostic significance has thus far not been evaluated.

Besides molecules of the major histocompatibility complex (MHC), adhesion molecules such as ICAM-1 seem to be important for facilitating a close direct contact between tumour cells and lymphocytes. ICAM-1 is a monomeric 90–114 kd glycoprotein that serves as a ligand for the lymphocyte function-associated antigen-1 (LFA-1) [11]. Cytokine-induced expression of ICAM-1 increases the vulnerability of tumour cells to monocyte- and T cell-mediated lysis [12, 13], and ICAM-1

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expression is critical for an effective HLA class II restricted T cell activation [14]. In contrast to these findings, *de novo* expression of ICAM-1 on melanoma cells is associated with an increased risk of metastasis [15].

In the present work, we assessed immunocytochemically the expression of HLA molecules and ICAM-1 on primary NSCLC, and evaluated its prognostic value. Our present results demonstrate a frequent coexpression of these immunological markers on primary NSCLC which, however, did not correlate with tumour recurrence or cancer-related survival.

PATIENTS AND METHODS

Patients and follow-up

Tumour specimens from 91 patients with NSCLC were examined. All patients underwent preoperatively the conventional staging procedures, and were found to have operable primary tumours (TNM stage less or equal to $T_3N_2M_0$). The median age at the time of operation was 59 years, range 38–77. In general, lobectomies or, if necessary, pneumonectomies were performed, followed by a radical lymphadenectomy. Patients, whose tumours were classified pathologically as $T_3N_2M_0$ or more after surgery, had adjuvant radiation therapy ($n=46$).

Follow-up care was organised by the department of pulmonary medicine. Evaluation included physical examination, chest X-ray and blood examinations every 3 months, and bronchoscopy, computed tomography (CT) scan of the thorax, abdominal ultrasound and a bone scan every 6 months after surgery. Complete information was obtained from a representative group of 78 patients, with a median follow-up of 22.6 months (range 6–35).

Monoclonal antibodies

HLA-A,B,C antigens were detected by the W6/32 antibody (IgG2a, kindly provided by D. Schendel, Institute of Immunology, Munich, Germany), which reacts with a monomorphic epitope of the HLA-A,B,C/β_2m complex [16]. TAL.1B5 (IgG1) was used to stain the 33 kd chain of the HLA class II complex (Dako, Hamburg, Germany) [17]. ICAM-1 was stained by the PA3.58-14 antibody (IgG2a), which was a gift from J. Johnson (Institute of Immunology, Munich, Germany) [18]. Tumour infiltrating leucocytes were detected by an antibody directed against the common leucocyte antigen (CD45, T29/33, IgG2b, Boehringer Mannheim, Germany). Localisation of the epithelial tumour cells within the specimen was confirmed by the anti-epithelial antibody EP4 (IgG1, Dako [19]), which stains more than 96% of all bronchogenic carcinomas (data not shown).

Tumour samples and immunohistochemistry

Representative tumour samples were obtained from the fresh surgical specimen, and immediately snap frozen in liquid nitrogen. The frozen tissue was stored at −80° until use. Of each frozen tumour sample, 10–15 serial cryostat sections of 5 μm thickness were cut and transferred on to glass slides. After air drying for 24 h, slides were stored at −20°.

For immunohistochemistry, the slides were fixed in acetone for 10 min and air dried for 45 min. Then, the cryostat sections were stained according to the APAAP staining procedure described by Cordell and colleagues [20]. Following rehydration through incubation with Tris-buffered solution (TBS, pH 7.6) for 15 min, AB serum (pooled serum from donors with blood group AB, diluted 1:10 with phosphate-buffered solution; Biotest, Germany) was applied for 20 min to block non-specific binding. Subsequently, specimens were incubated for 45 min with the primary antibody in appropriate dilutions. After each incubation step, the slides were washed three times with TBS. The bridging antibody (Dako) was applied for 30 min, followed by incubation with the APAAP complex (Dako) for 30 min. Staining was developed with fast red solution containing 1 mM levamisole for 15 min. Counter staining was performed with Meyer's haemalum solution. As a negative control for non-specific antibody binding the primary antibodies were replaced by irrelevant mouse myeloma proteins of identical isotypes (MOPC 21,IgG1;UPC 10, IgG2; Sigma, Deisenhofen, Germany). The cells from the tumour-surrounding stroma and cryostat sections of histopathologically metastases-free lymph nodes served as positive controls for ICAM-1 and HLA molecule expression.

Evaluation of the specimen

The slides were evaluated, double-blind, by two observers using light microscopy. HLA class I expression was considered to be lost if less than 10% of the tumour cells were stained. HLA class II and ICAM-1 expression was considered to be induced if more than 25% of the tumour cells expressed the antigen. To exclude false positive evaluation, the tumour-infiltrating leucocytes were identified on consecutive tumour sections by an anti-common leucocyte antigen antibody. In approximately 80% of the cases both observers obtained the same results; the remaining slides were re-evaluated and a consensus decision made.

Statistical analysis

For statistical analysis contingency tables were tested by a χ^2 test and, whenever appropriate Fisher's exact test was applied. For analysis of follow-up data, Kaplan–Meier curves were calculated and a log rank test was carried out.

RESULTS

Expression of HLA class I antigens

Deficient HLA-A,B,C expression (less than 10% positive tumour cells) was found in 33.0% of all types of bronchogenic carcinomas, with no significant difference between adenocarcinomas and squamous cell carcinomas (Table 1). However, four out of six adenosquamous carcinomas lacked HLA-A,B,C antigens.

Analysing the relationship between HLA class I expression and clinicopathological risk factors, we found that 22.0% of localised T_{1-3} adenocarcinomas had deficient HLA-A,B,C expression, in contrast to 75% of the T_4 tumours ($P = 0.04$, Table 2). There was, however, only a small number of T_4 tumours, and interpretation must be guarded. With regard to metastatic lymph node involvement, deficient expression of HLA class I in antigens was observed in 24.4 or 40% of all tumours from node-negative or from node-positive patients, respectively (Table 2). This difference was significant for patients with adenocarcinomas, with 10% (N_0) and 40% (N_{1-3}) HLA class I deficient, respectively ($P = 0.02$). Finally, deficient class I expression was more than twice as frequent in undifferentiated (G3) tumours than in well (G1) or moderately (G2) differentiated tumours ($P = 0.02$, Table 2). Interestingly, all of the above correlations with conventional risk factors were more pronounced in adenocarcinomas than in squamous cell carcinomas.

As shown in Table 3, tumours displaying normal levels of HLA class I antigens more frequently expressed HLA class II antigens ($P < 0.001$) and ICAM-1 ($P = 0.01$).

Table 1. Loss of HLA class I and expression of HLA class II antigens and ICAM-1 on different types of non-small cell lung carcinomas

	Adenocarcinoma (n = 45)	Squamous cell carcinoma (n = 37)	Adeno-squamous carcinoma (n = 6)	Large cell carcinoma (n = 3)	Total (n = 91)
HLA class I expression					
Normal	33 (73.3)‡	24 (64.7)	2	2	61 (67.0)
Deficient*	12 (26.7)	13 (35.1)	4	1	30 (33.0)
HLA class II expression					
Absent	31 (68.9)	28 (75.7)	5	3	67 (73.6)
Induced†	14 (41.1)	9 (24.3)	1	0	24 (26.4)
ICAM-1 expression‡					
Absent	26 (57.8)	32 (86.5)	6	0	64 (70.3)
Induced†	19 (42.2)	5 (13.5)§	0	3	27 (29.7)

*Less than 10% positive tumour cells. †More than 25% positive tumour cells. ‡Percentage of total number of patients per group. §Difference between adeno- and squamous cell carcinomas was significant with $P < 0.001$.

Table 2. Correlation of HLA class I, HLA class II and ICAM-1 expression on non-small cell lung carcinomas to T stage, N stage and tumour grading

		Deficient expression of HLA class I*	Neo-expression of HLA class II†	ICAM-1
Adenocarcinoma (n=45)				
T_{1-3}	(n = 41)	9 (22.0)‡§	14 (34.1)	19 (46.3)
T_4	(n = 4)	3 (75.0)	0	0
N_0	(n = 20)	2 (10.0)¶	9 (45.0)	9 (45.0)
N_{1-3}	(n = 25)	10 (40.0)	5 (20.0)	10 (40.0)
G_{1-2}	(n = 25)	4 (16.0)	8 (32.0)	11 (44.0)
G_3	(n = 20)	8 (40.0)	6 (30.0)	8 (40.0)
Squamous cell carcinoma (n=37)				
T_{1-3}	(n = 30)	10 (33.3)	8 (26.7)	5 (16.7)
T_4	(n = 7)	3 (42.9)	1 (14.3)	0
N_0	(n = 17)	5 (29.4)	4 (23.5)	2 (11.8)
N_{1-3}	(n = 20)	8 (40.0)	5 (25.0)	3 (15.0)
G_{1-2}	(n = 19)	6 (31.6)	4 (21.1)	3 (15.8)
G_3	(n = 18)	7 (38.9)	5 (27.8)	2 (11.1)
Total (n=91)				
T_{1-3}	(n = 80)	24 (30.0)	23 (28.8)	27 (33.8)**
T_4	(n = 11)	6 (54.5)	1 (9.1)	0
N_0	(n = 41)	10 (24.4)	13 (31.7)	12 (29.3)
N_{1-3}	(n = 50)	20 (40.0)	11 (22.0)	15 (30.0)
G_{1-2}	(n = 44)	10 (22.7)††	12 (27.3)	14 (31.8)
G_3	(n = 47)	20 (42.6)	12 (25.5)	13 (27.7)

*Less than 10% positive tumour cells. †More than 25% positive tumour cells. ‡Percentage of total number of patients per group. §Difference between T_{1-3} and T_4 is significant with $P = 0.04$. ¶Difference between N_0 and N_{1-3} is significant with $P = 0.02$. ‖All types of bronchiogenic carcinomas. **Difference between T_{1-3} and T_4 is significant with $P = 0.01$. ††Difference between G_{1-2} and G_3 is significant with $P = 0.02$.

Expression of HLA class II antigens

HLA class II antigens were expressed on 26.4% of all NSCLC carcinomas with little differences among the various tumour histologies (Table 1). Comparing the T stage of the patients with HLA class II antigen negative and positive tumours, we observed that T_{1-3} tumours appeared to express HLA class II antigens more oftenly than advanced T_4 tumours (28.8 versus 9.1%, respectively; Table 2). No significant correlations were found between the extent of lymph node involvement, the grade of differentiation of the tumour and HLA class II antigen expression (Table 2). However, the expression of HLA class II antigens on adenocarcinomas tended to be better correlated with T and N stage as compared to the respective expression on squamous cell carcinomas.

HLA class II antigens and ICAM-1 were frequently coexpressed on the same tumour (Table 3). Of the HLA class II antigen positive tumours 66.7% co-expressed ICAM-1, whereas only 16.4% of the HLA class II antigen negative tumours were ICAM-1 positive ($P < 0.01$).

Expression of ICAM-1

In total, 29.7% of the analysed bronchogenic carcinomas expressed ICAM-1 with a significant difference between adenocarcinomas (42.2%) and squamous cell carcinomas (13.5%) ($P < 0.001$; Table 1), while none of the six adenosquamous carcinomas was ICAM-1 positive. Interestingly, ICAM-1 expression on tumour cells was most prominent in areas with a marked infiltration of leucocytes.

Tumours of patients at a more advanced T stage were found to express ICAM-1 less frequently. Of 80 T_{1-3} tumours, 27 (33.8%) were ICAM-1 positive, whereas all 11 T_4 tumours lacked any detectable ICAM-1 expression ($P = 0.01$; Table 2). There was no correlation with the extent of lymph node involvement or the differentiation grade of the primary tumour (Table 2).

Survival analysis

The survival analysis calculated on the basis of 78 patients (Table 4) showed a significant difference in disease-free survival for the tumour stage (Fig. 1a). In contrast, we failed to detect any significant correlation between the expression of the HLA

Table 3. Coexpression of HLA class I, HLA class II antigens and ICAM-1 on non-small cell lung carcinomas

	HLA class I positive (n=61)	HLA class I negative (n=30)	HLA class II positive (n=24)	HLA class II negative (n=67)
ICAM-1 positive	23 (37.7)*,§	4 (13.3)	16 (66.7)†	11 (16.4)
ICAM-1 negative	38 (62.3)	26 (86.7)	8 (33.3)	56 (83.6)
HLA class II positive	24 (39.3)‡	0 (0)	—	—
HLA class II negative	37 (60.7)	30 (100)	—	—

*,†,‡Correlation between HLA class I, HLA class II and ICAM-1 expression was significant with $*P = 0.01$, $\dagger P < 0.01$, $\ddagger P < 0.001$. §Percentage of total number of patients per group are given in brackets.

Table 4. Influence of loss of HLA class I antigens and neo-expression of HLA class II antigens and ICAM-1 on tumour recurrence and survival

	No. of observations at risk	No. of tumour recurrences	No. of tumour-related deaths
Stage			
I	31	6 (19.3)*	4 (12.9)
II	10	5 (50.0, P=0.03)†	4 (40.0, P=0.02)
IIIa	28	17 (60.7, P<0.01)	13 (46.4, P<0.01)
IIIb	9	8 (88.8, P<0.01)	4 (44.4, P=0.02)
HLA class I expression			
Normal	54	26 (48.1)	16 (29.6)
Lost	24	9 (37.5)	8 (33.3)
HLA class II expression			
Absent	57	26 (45.6)	18 (31.6)
Induced	21	9 (42.9)	6 (28.6)
ICAM-1 expression			
Absent	52	24 (46.2)	17 (32.7)
Induced	26	11 (42.3)	7 (26.9)

*Percentage of total number of patients per group are given in parentheses. †P values in a log rank test versus stage I.

antigens or ICAM-1 and the clinical outcome of the overall group of patients (Fig. 1b–d). A more detailed analysis of subgroups of patients with different tumour stages, histologies or grades of differentiation also failed to reveal any significant prognostic relevance of the present immunostaining analysis (data not shown).

DISCUSSION

The ability of the immune system to recognise tumour cells as foreign is restricted through the expression of certain cell surface molecules. An increasing amount of experimental data demonstrate that, among those molecules, ICAM-1 and MHC antigens may play an important role. In the present study, we demonstrated that 33.0% of all bronchogenic carcinomas exhibited a deficient HLA-A,B,C expression (Table 1). The incidence of a reduced HLA-A,B,C expression in our series of bronchogenic carcinomas and the correlation with established risk factors is consistent with the recent results reported by Redondo and coworkers [9].

In a model of lung carcinoma in mice, it has been shown that the induction of class I antigens on tumour cells by interferon-γ renders them more sensitive to *in vivo* cytotoxic T cell lysis [21]. However, in our present investigation, we failed to demonstrate a significant prognostic influence of downregulation of HLA-A,B,C expression on human lung cancer cells. The absent correlation with tumour recurrence is consistent with the results reported on colorectal cancer [7, 22], whereas similar analysis of primary breast carcinomas or melanomas have indicated that patients with HLA-A,B,C negative tumours present with shorter survival times [6, 8].

The incidence of HLA class II positive tumours in our series of bronchogenic carcinomas was comparable to that reported on NSCLC [9], as well as other types of carcinomas [7, 23]. However, we failed to find any significant correlation between

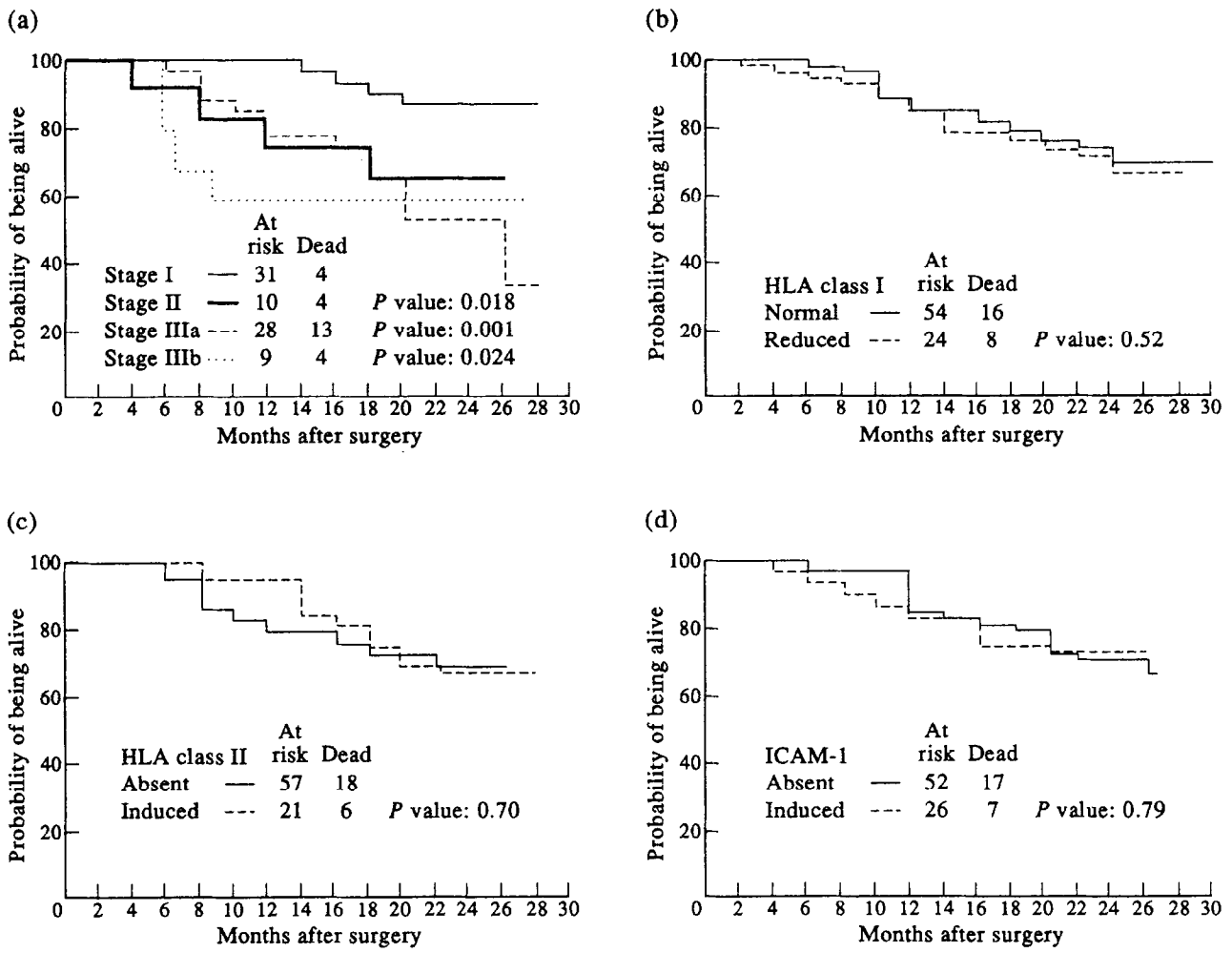

Fig. 1. Probability of survival in 78 patients with NSCLC according to tumour stage (a), mode of HLA class I antigen expression of the primary tumour (b), mode of HLA class II antigen expression of the primary tumour (c), mode of ICAM-1 expression of the primary tumour (d).

HLA class II expression and conventional risk factors or tumour recurrence. In contrast, Redondo and colleagues [9] described recently preferential expression of HLA class II antigens on well differentiated lung tumours, although follow up data was not provided.

The interaction of the immune cells with their targets is also influenced by additional molecules on the target cells, in particular by the cell adhesion molecules which interact with leucocyte ligands [24]. One of these molecules, ICAM-1, mediates leucocyte binding through its interaction with the integrins LFA-1 and Mac-1 (CD11b/CD18). We, therefore, tested bronchogenic carcinomas for neo-expression of ICAM-1. Our results indicate that neo-expression of ICAM-1 occurs preferentially in adenocarcinoma cells (42.2%), as compared to cells of squamous histology (13.5%, Table 1). The high frequency of ICAM-1 expression on bronchogenic adenocarcinomas is in contrast to the results reported by Natali and coworkers [25], who detected ICAM-1 in only one of 15 lung carcinomas. However, the different antibody and staining method used might explain this discrepancy. High incidences of ICAM-1-positive primary tumour cells have also been reported in renal cell carcinomas, while such expression is rare in colorectal or ovarian carcinomas [25, 26].

The biological and clinical significance of ICAM-1 expression by tumour cells is still under discussion. A series of experimental work supports the hypothesis that ICAM-1 may play a role in the specific recognition and destruction of tumour cells by cytotoxic lymphocytes. Cytokine-induced expression of ICAM-1 appears to increase the vulnerability of tumour cells to lysis by non-specific effector cells, such as monocytes [12], and ICAM-1 also seems to be required for effective activation of HLA class II restricted T cells [13].

Although our present study showed that ICAM-1 expression was preferentially observed at a less advanced tumour stage, no influence of such expression on tumour recurrence or survival was observed. Previous studies on renal cell carcinomas have demonstrated a correlation with well differentiated primary tumours as a parameter for a more favourable prognosis [26]. In contrast, neo-expression of ICAM-1 on human melanoma cells and high levels of circulating ICAM-1 in serum was associated with an increased risk of metastasis and a poor clinical outcome [15, 27]. Thus, the prognostic significance of ICAM-1 expression by primary tumour cells seems to depend on the type of tumour.

Coexpression of ICMA-1 and HLA class II on consecutive tumour specimens from the same patient was frequently observed, suggesting a co-ordinated expression of ICAM-1 and HLA class II molecules. A possible explanation for this

observation is that tumour cells may use common regulatory pathways to stimulate the synthesis of these molecules, such as the induction by interferon-γ and tumour necrosis factor-α. The presence of receptors for these substances on tumour cells may, therefore, determine the simultaneous expression of HLA antigens and ICAM-1. In this context, it is noteworthy that we observed the strongest anti-ICAM-1 monoclonal antibody reactivity in tumour areas heavily infiltrated with leucocytes. This suggests that expression of ICAM-1 on primary lung tumour cells might be induced or amplified by inflammatory cytokines released from tumour infiltrating leucocytes. The significance of tumour infiltrating lymphocytes for the expression of immunoregulatory molecules by malignant epithelial cells is discussed controversely. In breast cancer, Göttlinger and colleagues [28] reported a strong association between T cell content of the tumour and MHC class II expression in a study, while other authors failed to establish such a correlation [29, 30].

In conclusion, the expression of HLA antigens and ICAM-1 on primary NSCLC appears to have no influence on tumour recurrence and survival. Nevertheless, these molecules might be important for the survival and outgrowth of individual disseminated tumour cells, as suggested by our recent investigations [4, 31].

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