Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Immunology Letters, 42 (1994) 19–23
Elsevier Science B.V.

IMLET 2205

Expression of aminopeptidase N/CD13 in tumour-infiltrating lymphocytes from human renal cell carcinoma

Dagmar Riemann [a,*], Barbara Göhring [b] and Jürgen Langner [a]

[a] *Institute of Medical Immunology, Martin Luther University, 06097 Halle, Germany and* [b] *Department of Urology, Martin Luther University, 06097 Halle, Germany*

(Received 15 June 1994; accepted 21 June 1994)

Key words: Aminopeptidase N; CD13; Renal cell carcinoma; Tumour-infiltrating lymphocytes

1. Summary

We have previously demonstrated the expression of aminopeptidase N (APN, CD13) on synovial T cells from patients with different forms of arthritis. T cells of peripheral blood and serous body fluids are CD13-negative but can be stimulated to express CD13 after activation, e.g., with Con A. In the present report, double-labelling and flow cytometry analyses were performed to characterize the phenotype of tumour-infiltrating lymphocytes (TIL). A large panel of antibodies specific for different activation-associated molecules on T cells was used. In contrast to TIL of lung cancer, TIL of renal cell carcinoma (RCC) consisted of significantly higher percentages of T cells expressing CD13, dipeptidylpeptidase N (DPIV, CD26) and HLA-DR, whereas T cells of lung cancer expressed more CD25, CD69 and CD54/ICAM1. No differences could be found in the expression of CD45RO, CD49a/VLA-1 and CD62L/L-selectin. Our results demonstrate that T cells in RCC and lung cancer differ in their phenotype, especially with respect to surface aminopeptidases. Investigations into the function of APN on T cells could be of help in gaining deeper insight into tumour defence as well as into general mechanisms of T cell functions.

2. Introduction

Cell surface peptidases play an important role in signal transduction and communication between cells. A great number of studies have been published with respect to the participation of dipeptidylpeptidase IV (DPIV, EC 3.4.14.5) in T cell activation and memory [for review see 1,2]. Aminopeptidase N (APN, EC 3.4.11.2), a surface antigen of many cells, among them small intestinal and renal tubular epithelial cells, and also of synaptic membranes, acts with a relatively broad substrate specificity on peptides with an N-terminal neutral amino acid. Sequence comparisons of the cloned cDNA showed that APN was identical to CD13 [3]. Though on haematopoietic cells CD13 has been considered to be a myeloic-specific cell marker, CD13 specific RNA has been detected also in T cell lines [4]. Furthermore CD13 surface expression has been described in T cell cultures expanded with IL-2 or IL-4 [5,6], in cutaneous T cell lymphoma [7] and in T cells in inflamed tissues [8]. The significance of these findings remains unknown. In the present paper we compare the T cell phenotype of lung cancer and renal cell carcinoma (RCC) using a broad panel of antibodies specific to activation-associated molecules. We describe for the first time the occurrence of $CD13^+$ tumour-infiltrating lymphocytes (TIL) especially from RCC.

* *Corresponding author:* D. Riemann, The Institute of Medical Immunology, Martin Luther University, Halle-Wittenberg, Straße der OdF 6, 06097 Halle, Germany. Tel: 0345/671358; Fax: 0345/671357.

Abbreviations: APN, aminopeptidase N; RCC, renal cell carcinoma; DPIV, dipeptidylpeptidase IV; TIL, tumour-infiltrating lymphocytes.

3. Materials and Methods

3.1. Tumours

114 human tumours of various histologies, including 37 renal cell carcinomas, 20 adenocarcinomas (lung), 37 squamous cell carcinomas (lung), 10 metastases at the lung and 10 tumours of various localizations, were removed at the Surgery Branch of the Martin Luther University, Halle-Wittenberg. Following surgery, the tissues were harvested in a sterile antibiotic-containing

TABLE 1
CELLULAR COMPOSITION OF TIL ISOLATED FROM SQUAMOUS CELL CARCINOMA (LUNG CANCER, GROUP 1), ADENOCARCINOMA (LUNG CANCER, GROUP 2) AND RENAL CELL CARCINOMA (GROUP 3)

		% T cells $CD3^+$	% B cells $CD19^+$	% NK cells $CD3^-$ $CD16^+ + CD56^+$	Ratio CD4:CD8
group 1	range	48–97	1–42	0–30	0.5–4.9
($n = 37$)	mean	76	16	6	1.8
group 2	range	45–95	2–50	0–37	0.7–3.4
($n = 20$)	mean	74	18	6	1.7
group 3	range	40–95	0–35	2–37	0.5–5.9
($n = 37$)	mean	80	6	12	1.6

medium and processed within 6 h. Samples of tumour were sent for routine histopathology.

3.2. Preparation of tumour-infiltrating lymphocytes

Solid tumours were dissected into 5 mm^3 pieces which were immersed in RPMI 1640 medium containing collagenase type IV 0.1%, DNAse type I 0.015% (Sigma, Deisenhofen, Germany). This mixture was stirred for 1–2 h at 37°C. It was then filtered through a coarse wire grid to exclude undigested tissue fragments. The resultant cell suspension was washed twice in PBS. Mononuclear cells were separated by Ficoll-Hypaque gradient centrifugation and washed twice more with PBS.

3.3. Phenotypic analysis

Cells were resuspended in a concentration of 1×10^6 cells/ml. Double staining was performed at 20°C for 20 minutes. After two cold washes with PBS containing 0.1% sodium azide, the cells were fixed using 1% paraformaldehyde. Fluorescence analyses were performed on a Becton Dickinson FACScan. Non-lymphoid and dead cells were excluded by the setting of appropriate forward and 90° light scatter gates. 2000 cells per lymphocyte gate were counted. The threshold was defined in such a manner that positive-staining included no more than 1% of the relevant negative control.

3.4. Monoclonal antibodies

The monoclonal antibodies (FITC/Phycoerythrin) used were CD45/CD14 (leucogate), the negative control, CD3/CD19, CD4/CD8, CD3/HLA-DR, CD3/CD16 + CD56 (Simulset kit, Becton Dickinson), CD3/CD25, CD3/CD13, CD3/CD11b, CD3/CD45RO, CD3/L-selectin, CD3/CD54, CD3/CD69, CD26/CD3 and CD49a/CD3. The antibody for CD26 was purchased from Coulter (Krefeld, Germany), the CD25-antibody was obtained from Dianova (Hamburg, Germany), the CD49a-antibody was from T cell Sciences (Cambridge). All other antibodies were from Becton Dickinson. Optimal dilutions for use in flow cytometry were established in preliminary experiments.

3.5. Statistical analyses

Data are expressed as range or as mean ± standard deviation and the Wilcoxon rank sum test was used to determine whether two experimental values were significantly different ($P < 0.01$).

4. Results

Table 1 presents the cellular composition of TIL of lung squamous cell carcinoma, lung adenocarcinoma and RCC. T cells dominated among TIL of the three

TABLE 2
EXPRESSION OF ACTIVATION-ASSOCIATED MOLECULES ON $CD3^+$ TIL ISOLATED FROM LUNG CANCER (GROUP 1 = SQUAMOUS CELL CARCINOMA; GROUP 2 = ADENOCARCINOMA) AND RCC (GROUP 3).

		HLA-DR	CD25	CD13	CD26	CD45RO	CD54	CD62L	CD49a	CD69	CD11b
group 1	range	18–82	9–40	2–41	18–63	91–96	1–56	2–29	7–61	53–98	1–41
($n = 37$)	mean ± SD	48 ± 15	22 ± 8	11 ± 9	40 ± 10	93 ± 5	19 ± 12	11 ± 8	33 ± 12	87 ± 8	15 ± 9
group 2	range	25–78	7–42	3–30	25–72	81–99	2–50	0–20	14–57	53–99	8–58
($n = 20$)	mean ± SD	48 ± 15	20 ± 8	14 ± 8	46 ± 13	93 ± 4	21 ± 13	8 ± 5	33 ± 13	87 ± 10	21 ± 13
group 3	range	19–84	3–19	7–88	48–100	66–98	0–40	2–25	7–90	27–94	8–62
($n = 37$)	mean ± SD	62 ± 16	9 ± 3	33 ± 24	62 ± 13	89 ± 7	14 ± 10	8 ± 5	29 ± 17	64 ± 16	28 ± 12

Results are given as a percentage of $CD3^+$ cells, values are expressed as range and mean ± SD.

tumour histologies. The percentage of B cell was significantly higher in lung cancer than in RCC. Natural killer cells constituted only a minority of TIL, but were significantly higher in RCC compared with lung cancer. The ratio CD4 : CD8 showed no differences within all groups.

Table 2 demonstrates the results of the phenotyping of T cells in lung cancers and RCC. In contrast to T cells in peripheral blood, > 90% of T cells isolated from tumours expressed CD45RO. T lymphocytes from RCC had the highest percentages of $CD13^+$, $CD26^+$ and $HLA\text{-}DR^+$ cells and the lowest numbers of $CD25^+$, $CD69^+$ and $CD54^+$ cells. We found no significant difference in the expression on $CD3^+$ cells of CD45RO, CD49a/VLA-1 and CD62/L-selectin. The only difference in the T cell phenotype between adenocarcinoma and squamous cell carcinoma of the lung was observed with respect to CD11b expression of T cells. TIL of squamous cell carcinoma contained significantly fewer $CD11b^+$ T cells than TIL of RCC and adenocarcinoma of the lung. Fig. 1 illustrates one example of double-staining of RCC TIL with CD4/CD13 and CD8/CD13. Both T cell subpopulations expressed CD13 as already found with synovial fluid T lymphocytes [8]. Table 3 shows examples for the phenotyping of TIL of different lung metastases as well as of tumours of other localizations with respect to the expression of HLA-DR, CD25, CD13 and CD26. One of three lung metastases of RCC had a high percentage of $CD13^+$ T cells. This demonstrates that also within the lung TIL can contain high proportions of $CD13^+$ T cells. We further found that one patient with breast cancer and one with sarcoma had high numbers of $CD13^+$ T cells. This indicates that CD13 expression on tumour-infiltrating T cells is not exclusively a characteristic property of RCC. Further investigations with a greater panel of different tumour types are in preparation to clarify this point.

TABLE 3
EXPRESSION OF FOUR SELECTED SURFACE MOLECULES ON TIL OF LUNG METASTASES AND OF TUMOUR SAMPLES OF VARIOUS LOCALIZATIONS

I lung as metastatic tumour site

patient	tumour	HLA-DR	CD25	CD13	CD26
S.H.	melanoma	46	14	7	51
K.K.	gastric cancer	57	30	8	29
S.R.	breast cancer	68	9	38	79
M.I.	breast cancer	48	9	15	41
B.R.	breast cancer	52	24	10	54
R.G.	colon cancer	29	10	17	62
T.E.	RCC	84	12	73	82
H.I.	RCC	32	19	18	62
V.H.	RCC	63	18	10	46
K.P.	carcinoma of the larynx	24	20	13	35

II tumours of other localizations

patient	tumour	HLA-DR	CD25	CD13	CD26
A.L.	gastric cancer	68	40	18	46
L.H.	colon cancer	30	29	14	40
F.E.	gastric cancer	41	15	10	34
T.G.	gastric cancer	31	9	4	50
W.R.	gastric cancer	71	25	21	50
T.I.	breast cancer	36	11	15	53
P.H.	breast cancer	59	10	7	40
F.R.	hysterocarcinoma	16	11	3	58
K.L.	hysterocarcinoma	40	11	14	46
S.K.	sarcoma	67	24	59	70

Results are given as a percentage of $CD3^+$ cells.

5. Discussion

The data presented in this paper indicate that TIL of lung cancer (adenocarcinoma as well as squamous cell carcinoma) differ from TIL of renal cancer with respect to the expression of T cell surface molecules such as HLA-DR, CD69, CD54 and CD25 but also surface aminopeptidases such as DPIV/CD26 and APN/CD13. No difference could be found for the expression of

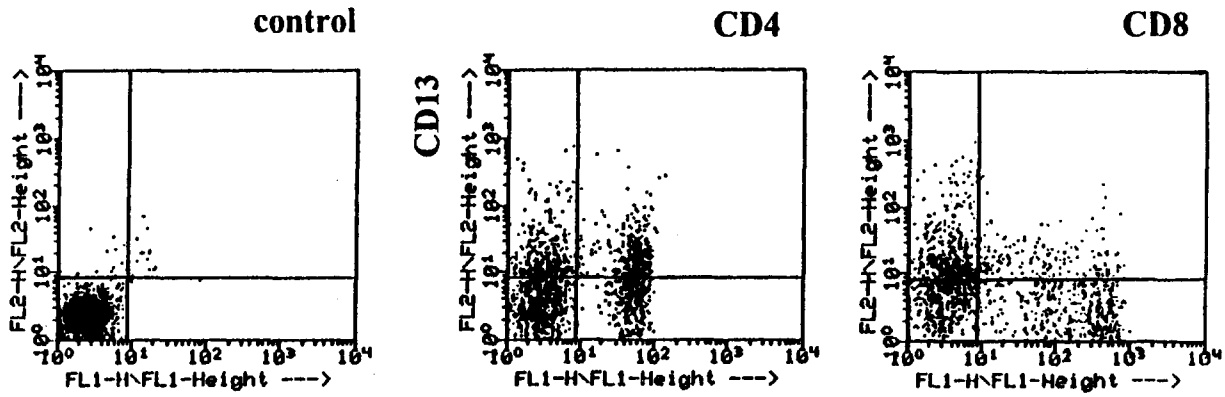

Fig. 1. Two-colour immunofluorescence analysis of TIL from RCC.

CD45RO and adhesion molecules such as CD49a (β_1 subfamily of integrins), CD11b (β_2 subfamily) and CD62L (selectin family of adhesion proteins). At present, both the reason for and the consequences of the observed differences remain unknown. Especially the high percentage of $CD13^+$ T cells in RCC seems to be a very interesting finding. T cells in peripheral blood are $CD13^-$. Our first report on $CD13^+$ T cells in the synovial fluid of patients with different forms of arthritis [8] suggested a role for APN within the special conditions of an inflamed joint. In subsequent investigations [9], we observed varying levels of $CD13^+$ T cells in the pericardial fluid of patients undergoing heart valve replacement. These patients often had endocarditis in their medical history. Additionally, we always found only small CD13 expression on T cells from bronchoalveolar lavage fluid from patients with inflammatory lung diseases [10]. Now we are able to show that TIL can also express CD13, though differences exist between histologically distinct tumours and different patients. CD13 showed a broad range of expression of between 7 and 88% of $CD3^+$ cells especially in RCC but not in lung cancer.

Lymphocytic infiltration is a consistent component for certain tumours, and lymphoid infiltrates in or around tumours reveal similarities with a "chronic inflammation". TIL are a heterogeneous population of cells. Whether $CD13^+$ TIL in RCC represent a special lymphocytic subpopulation with its own migratory behaviour remains unknown. The fact that $CD13^+$ T cells also occur in the inflamed joint and in some pericardial fluids seems to favour our hypothesis that $CD13^+$ T cells are the result of a special "inflammation-associated" event. This hypothesis is supported in so far as RCC is a highly immunogenic tumour with reported cases of spontaneous regression of metastatic lesions [11]. $CD13^+$ T cells could have passed through a kind of maturational process and CD13 expression on T cells could be associated with a special functional capacity. With respect to these considerations, it is interesting that T cells in synovial fluid as well as in RCC contain a high number of $HLA\text{-}DR^+$ but a small amount of $CD25^+$ cells. T cells in RCC have been reported to secrete cytokines of the Th2 type [12]. IL-4, a cytokine with tumour growth-inhibiting properties [13], can induce CD13 expression on monocytes/macrophages [14] and — according to our own observations — also on lymphocytes [6]. If IL-4 is really the CD13 inducing cytokine in RCC, one could speculate that TIL in RCC and lung cancer differ in their cytokine secretion, a point that needs further investigation. Additionally, studies are in preparation to compare TIL from different RCC samples with small or high percentages of $CD13^+$ T cells with respect to T cell functions.

Our knowledge of the myeloic marker APN/CD13 on T cells is still in its early stages. As a membrane peptidase, APN seems suitable for regulation of peptide hormones. This has already been shown for enkephalins and endorphins degraded by APN in synaptic membranes of the central nervous system [15]. Additionally, it has been suggested that APN plays a role in antigen processing [16] and in cell adhesion [17,18]. Furthermore, the observation that CD13 represents a receptor for corona viruses could be of major pathological importance [19,20]. Studies on the peptide substrates and the regulation of APN/CD13 on lymphocytes will provide additional insight into lymphocytic functions under special activation-associated situations.

Acknowledgements

We would like to thank the Department of Surgery, Urology and the Institute of Pathology of the Martin Luther University, for their co-operation. We acknowledge the technical assistance of Rosemarie Meinicke and Karin Jung and the expert secretarial assistance of Christel Walcker. This work was funded in part with grants from the Deutsche Forschungsgemeinschaft (Langner 740/2-1) and from the BMFT (01ZZ9105, part XIII).

References

[1] Marguet, D., David, F., Vivier, I., Bernard, A.M., Naquet, P. and Pierres, M. (1993) Adv. Neuroimmunol. 3, 209.
[2] Fleischer, B. (1994) Immunol. Today 15, 180.
[3] Look, A.T., Ashmun, R.A., Shapiro, L.H. and Peiper, S.C. (1989) J. Clin. Invest. 83, 1299.
[4] Lendeckel, U., Wex, T., Kähne, T., Frank, K., Reinhold, D. and Ansorge, S. (1994) Cell. Immunol. 153, 214.
[5] Kan-Mitchell, J., Huang, X.Q., Steinman, L., Oksenberg, J.R., Harel, W., Parker, J.W., Goedegebuure, P.S., Darrow, T.L. and Mitchell, M.S. (1993) Cancer Immunol. Immunother. 37, 15.
[6] Riemann, D., Göhring, B., Kehlen, A. and Langner, J. (1993) Immunobiol. 189, 162.
[7] Dreno, B., Bureau, B., Stalder, J.F. and Litoux, P. (1990) Arch. Dermatol. 126, 145.
[8] Riemann, D., Schwachula, A., Hentschel, M. and Langner, J. (1993) Immunobiol. 187, 24.
[9] Riemann, D., Wollert, H.G., Menschikowski, J., Mittenzwei, S. and Langner, J. (1994) Int. Arch. Allergy Immunol. 104, 48.
[10] Riemann, D., Schwachula, A. and Langner, J. (1993) Clin. Rheumatol. 12, 31.
[11] Nakano, E., Sonoda, T., Fujioka, H. (1984) Eur. Urol. 10, 212.
[12] Schoof, D.D., Terashima, Y., Peoples, G.E., Goedegebuure, P.S., Andrews, J.V.R., Richie, J.P. and Eberlein, T.J. (1993) Cell Immunol. 150, 114.
[13] Hoon, D.S.B., Okun, E., Banez, M., Irie, R.F. and Morton, D.L. (1991) Cancer Res. 51, 5687.
[14] Van Hal, P.T., Hopstaken-Broos, J.P.M., Wijkhuijs, J.M.,

TeVelde, A.A., Figdor, C.G. and Hoogsteden, H.C. (1992) J. Immunol. 149, 1395.
[15] Matsas, R., Stephenson, S.L., Hryszko, J., Kenny, A.J. and Turner, A.J. (1985) Biochem. J. 231, 445.
[16] Stryhn Hansen, A., Norén, O., Sjöström, H. and Werdelin, O. (1993) Eur. J. Immunol. 23, 2358.
[17] Saiki, I., Fujii, H., Yoneda, J., Abe, F., Nakajima, M., Tsuruo, T. and Azuma, I. (1993) Int. J. Cancer 54, 137.
[18] Menrad, A., Speicher, D., Wacker, J. and Herlyn, M. (1993) Cancer Res. 53, 1450.
[19] Delmas, B., Gelfi, J., L'Haridou, R., Vogel, L.K. Sjöström, H., Norén, O. and Laude, H. (1992) Nature 357, 417.
[20] Yeager, C.L., Ashmun, R.A., Williams, R.K., Cardellichio, C.B., Shapiro, L.H., Look, A.T. and Holmes, K.V. (1992) Nature 357, 420.