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

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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.

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...
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 mm³ 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 X 10⁶ 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 groups.

| Group | % 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 |

Table 2 presents the expression of activation-associated molecules on CD3⁺ TIL isolated from lung cancer (Group 1 = squamous cell carcinoma; Group 2 = adenocarcinoma) and RCC (Group 3).

| Group | 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-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**

| Patient | Tumour Type  | 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.L.    | RCC           | 32     | 19   | 18   | 62   |
| V.H.    | RCC           | 63     | 18   | 10   | 46   |
| K.P.    | carcinoma of the larynx | 24 | 20 | 13 | 35 |

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...
CD45RO and adhesion molecules such as CD49α (β₁ subfamily of integrins), CD11b (β₂ 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 endocardi-tis 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-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.

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