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Markers of peripheral blood lymphocyte activation in local and advanced gatrointestinal cancer patients

Simona Donina[1],

Inta Jaunalksne[2*],

Ludmila Engele[1],

Tatjana Smirnova[1],

Tatjana Romanova[2]

[1] *Latvian Oncology Centre, Riga, 4 Hipokrata Str. LV-1079, Latvia*

[2] *VAS Paula Stradina KUS Clinical Immunology Center, Pilsonu 13, LV-1002 Riga, Latvia*

Bacground. Peripheral blood (PB) immunocompetent cells play an important role in antitumour immune response. According to markers expressed on PB cells, these cells may go to apoptosis or induce immune response.

Materials and methods. We examined 46 gastrointestinal (GI) cancer patients at various stages of disease with the aim to determine $CD3^+$, $CD4^+$, $CD8^+$, $CD38^+$, $CD95^+$, CD95L and $CD25^+$ cells in PB and sIL2R, TNFa and sTNFRI in serum.

Results. The results showed an insufficiency of the inductor phase of T cell immune response due to a decreased number of $CD4^+$ cells in advanced gastrointestinal cancer patients and compensatory activity of effector cells.

Elevated levels of sIL2R and an increased number of $CD95^+$ cells were observed in advanced gastrointestinal cancer patients. The level of TNFa and sTNFRI did not differ significantly in patients with local and advanced gastrointestinal cancer. **Conclusions**. The variability of apoptotic and activation marker expression of PB immunocompetent cells shows a difference in immune response at various stages of gastrointestinal cancer. Cellular or serological markers of PB immune cell activation may be useful for under-standing an individual reaction to treatment and for the prognosis of disease.

Key words: gastrointestinal cancer, PB lymphocytes (ly), activation markers

INTRODUCTION

Although the immune system is able to recognize and kill tumor cells through its lymphoid effectors, as shown by the findings of both *in vitro* and *in vivo* studies (1), it is well known that neoplastic disease has a profound influence on immunological functions, and cancer patients generally have an inadequate antitumour response (2). Probably several mechanisms underlie the lack of efficient spontaneous immune reactions observed in cancer patients. A growing tumour burden has been associated with a decreasing immunocompetence, manifesting the development of lymphopenia and disturbances in the network of cells for immune regulation (3). Disbalance between the immune cells' ability to proliferate and to go to apoptosis leads to deviations of cellular immune response.

Antigen-presenting cells introduce the antigen peptide by the major histocompatibility complex to $CD4^+$ cells and iniciate immune response by producing cytokines IL2, IFNγ, TNF, which influence activity of effector cells. Activated effector cells may become CD25, CD38 positive as well as CD95 and CD95L positive (4).

* Author for correspondence. Fax: 371 706 9259.

According to markers expressed on peripheral blood (PB) immunocompetent cells, these cells may go to apoptosis or induce immune response.

IL2, defined as a growth factor, shows pleiotropic activities on cell-mediated and humoral immunity. IL2 improves T cell proliferation, increases the generation of cytotoxic T ly and induces activation of T and B cells (5). Moreover, IL2 increases the antitumour activity of natural killer (NK) cells (6) to realize the functions mentioned above; IL2 has to bind a corresponding receptor on the T cell surface.

During activation, IL2 receptors (IL2R) are cleared from the cell surface and a soluble form of IL2R is produced (7). The release of sIL2R into biological fluids is one of the most sensitive indicators of the presence of activated T ly *in vivo* (8).

Tumour necrosis factor alpha (TNFα) has numerous biological functions such as induction of hemorrhagic necrosis of transplanted tumours, development of inflammatory response and immunoregulatory functions. TNFα initiates its multiple functions by binding to two specific, high-afinity receptors and has been involved in Fas-mediated cell apoptosis through binding to the tumour necrosis factor receptor I (TNFRI). Cleavage products of the extracellular domain of

membrane-bound TNF receptors (sTNFRI and sTNRII) are found in the serum and urine (3, 9, 10, 11) of humans. Because of their ability to compete with the cell surface form of TNF receptor, these soluble forms (as report *in vitro* studies) may function as an inhibitor of TNF bioactivity (12, 13).

Fas receptor (CD95) and Fas-ligand (CD95L) are the main systems that regulate immune response. Fas (CD95) is a member of the TNF cell surface receptor family, and the ligation of a surface membrane molecule CD95 on activated T cells by its ligand leads to apoptosis of Fas receptor bearing cells (4, 14, 15). *In vitro* studies suggest that Fas preferentially controls the death of $CD4^+$ T cells. Fas R and Fas-L expression is upregulated after ly activation (16).

The majority of data reflect expression of activation markers of immune cells as well as the proliferation and apoptotic processes of these cells *in vitro*.

With the aim to obtain a complicated immune reaction *in vivo*, we examined gastrointestinal cancer patients' peripheral blood immunocompetent cells for markers of all activation and apoptosis in various stages of disease because of different course of disease and treatment options.

MATERIALS AND METHODS

Forty-six gastrointestinal cancer patients with histologically confirmed adenocarcinoma were examined before surgery. None of these patients had received radiotherapy, chemotherapy or immunotherapy before operation. The characteristics of patients are shown in Table 1.

Table 1. **Characteristics of patients**

Sex	Male Female	21 25
Age	Range Average	36–76 56
Tumour differentiation	Good Moderate Poor	8 26 12
TNM stage	I + II III + IV	4 + 16 17 + 9
Total		46

Whole blood samples were collected for flow – cytometry into vacutainers with EDTA. Leukocytes were isolated by lysis of red cells in ammonium chloride lysine buffer. PB ly were then separated by centrifugation at room temperature and washed twice with PBS.

Ly subsets were determined by a FACS Calibur laser flow cytometer (Becton-Dickinson, USA) using monoclonal antibodies to $CD3^+$, $CD4^+$, $CD8^+$, $CD38^+$, $CD95^+$, $CD95L^+$ and $CD25^+$ cells.

The patients' serum was collected for an automatic hemiluminescence analyzer Immulite (DPC, USA) to determine sIL2R, TNFα and sTNFRI by the ELISA method and Biosource sTNFRI kit EASIA (Belgium).

Statistical analysis was performed using the Student's t test, and a p value 0.05 was taken to be statistically significant.

RESULTS

The absolute number of $CD4^+$ cells was significantly lower in patients with advanced gastrointestinal cancer (stages III and IV according to TNM classification) as compared to patients with local disease (stages I and II according to TNM classification). In contrast to $CD4^+$ cells, the absolute number of $CD8^+$ cells was higher in the advanced gastrointestinal cancer group (Table 2).

Table 2. **Absolute number (cells/mm^3) of $CD3^+$, $CD4^+$, $CD8^+$ positive cells in gastrointestinal cancer patients**

	Stage I + II	Stage III + IV
$CD3^+$	1420 ± 663	1286 ± 491
* $CD4^+$	805 ± 212	630 ± 178
* $CD8^+$	403 ± 162	548 ± 192

* $p < 0.05$

A tendency to a decrease was observed in the amount of T ly of PB when the malignant process was spread.

A comparison of the absolute numbers of activated lymphocytes determined from gastrointestinal cancer patients with local and advanced disease was made. No significant difference in the number of $CD25^+$ and $CD38^+$ cells was observed (Table 3).

Table 3. **Absolute number (cells/mm^3) of $CD25^+$, $CD38^+$ in gastrointestinal cancer patients**

	Stage I + II	Stage III + IV
$CD25^+$	42 ± 28	36 ± 20
$CD38^+$	514 ± 178	532 ± 234

Although the number of IL2-receptor-bearing cells did not differ in the observed groups, the level of soluble IL2 receptors was increased in the serum of advanced gastrointestinal cancer patients in comparison to those with local disease (604 ± 201 U/ml and 437 ± 151 U/ml, respectively).

The absolute number of Fas-receptor-bearing T cells in peripheral blood was significantly higher in patients when gastrointestinal cancer metastases had been formed in lymphocytes or visceral organs.

In contrast to $CD95^+$ cells, the absolute number of Fas-ligand-bearing cells ($CD95L^+$) did not change significantly in either of the groups (Table 4).

Table 4. **Absolute number (cells/mm^3) of CD95$^+$, CD95L$^+$ cells in gastrointestinal cancer patients**

	Stage I + II	Stage III + IV
* CD95$^+$	587 ± 216	740 ± 280
CD95L$^+$	107 ± 31	117 ± 39

* $p < 0.05$

The level of TNF$^+\alpha$ was similar in patients with local and advanced gastrointestinal cancer, but the sTNFRI level in serum was slightly elevated in patients with advanced disease (Table 5).

Table 5. **TNFα and sTNFRI (pg/ml) in serum of gastrointestinal cancer patients**

	Stage I + II	Stage III + IV
TNFα	5.6 ± 1.2	5.8 ± 1.2
sTNFRI	4.1 ± 0.9	4.8 ± 1.0

DISCUSSION

As previously reported, cancer patients are generally immunocompromised and T cell functions in them are often insufficient (2, 17, 18). Our data showed a depression of the inductor phase of T cell immune response reflected in a decreased number of CD4$^+$ cells in gastrointestinal cancer patients in advanced stages. Although the number of CD4$^+$ cells was decreased, a relatively high expression of the activation markers CD25 and CD38 was observed in advanced stages of gastrointestinal cancer. These data allow us to suggest a compensatory ly activation in pretreated patients (as long as tumor antigen stimulation is present) with the aim to support the effector phase of cellular immune response. Due to cellular activation, an increasing number of effector cytotoxic T ly (CD8$^+$ cells) was observed in patients with advanced disease in our study.

Interaction of IL2 with its specific receptor leads to pleotropic activities of immune response. The serum level of IL2 is mentioned as a factor associated with advanced cancer and poor prognosis, and a soluble form of IL2 receptors releasing in serum may be an indicator of the course of disease too. The sIL2 receptor may bind circulating IL2 and influence immune response (3, 19, 20). Our data are similar and show a high level of sIL2R in advanced gastrointestinal cancer patients' serum, confirming a probability of T cell activation in patients' PB simultaneously with insufficient probability to induce immune response by blocking the action of IL2 in serum.

According to the literature, several tumor types, including primary colorectal carcinoma and hepatocellular carcinoma, express Fas-L and exploit its ability to kill Fas-bearing ly to evade immune recognition and contribute to T cell damage and apoptosis (17, 21, 22). Expression of Fas-receptors on cell surface is stable when the cell is activated, and the cell may become Fas-L sensitive (21).

The data obtained in this study suggest that antigen stimulation may lead to an overexpression of Fas-receptors on T ly and that the number of CD95$^+$ cells increases with the spread of the malignant process. Some results indicate that Fas-L is upregulated in non-lymphoid cells during immune reaction, and it seems also to cause deletion of the activated T cells (23). Our results did not show overexpression of Fas-L in advanced gastrointestinal cancer patients' ly as compared to those with local disease, and it may indicate that advanced cancer patients are more sensitive to Fas-mediated apoptosis and have less ability to induce apoptosis in cancer cells. TNFα and TNFRI are involved in Fas-mediated ly apoptosis and can also contribute to ly death (24).

Some authors reported increased levels of sTNFRI in gastrointestinal cancer patients' serum correlating with the advance of disease, a negative correlation with CD3$^+$ cells and a positive correlation with sIL2R (3). However, sTNFRI may block some functions of TNFα (ability to induce activation of macrophages, influence of Fas-mediated apoptosis, etc.). Our data did not reflect the importance of TNFα and sTNFRI in PB cell apoptotic process during gastrointestinal cancer progression. This small observation allows us to suggest that not always data obtained *in vitro* exactly reflect immune reactions *in vivo*, and *in vivo* reactions are very individual and show only tendencies of changes in the immune parameters and immune response.

CONCLUSIONS

Advanced gastrointestinal cancer is associated with a decreased number of CD4$^+$ cells in PB, which may lead to a depression of cellular immune response.

Compensatory activation of PB ly was observed in the group of advanced gastrointestinal cancer patients.

PB ly in advanced gastrointestinal cancer patients are more sensitive to Fas-mediated apoptosis as compared to cases of local disease.

The differences in activation of PB cells and expression of apoptotic markers show differences in immune response in patients with local and advanced gastrointestinal cancer.

Cellular or serological markers of PB immune cell activation may be useful for understanding an individual reaction to treatment and the prognosis of disease.

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