Epidemiological studies prove that incidence of colorectal cancer is increasing. The first line therapy of colorectal cancer is surgical resection of the primary tumor and elimination of regional and remote metastases.

**The aim of the study** was to determine expression of adhesion molecules CD134 and CD137 in the peripheral blood in colorectal cancer patients, depending on clinical cancer stage, size and invasion of the tumor.

**Material and methods.** The study enrolled 72 patients with primary colorectal adenocarcinoma. An average patient age was 64.55 years. Clinical tumor stage was assessed using two scales: Dukes: A and Astler-Coller scale. Expression of adhesion molecules was determined in the peripheral blood collected on the day of the procedure and 10 days after the procedure.

**Results.** An average activity of CD134 molecules (12.66%) was significantly higher than that of CD137 (6.26%) (p<0.001). Clinical tumor stage was assessed on Dukes scale and was unrelated to CD134 activity, while activity of CD137 was related to clinical cancer stage.

**Conclusions.** CD137 activity is directly proportional to colorectal cancer stage. Surgical resection of the tumor results in increased CD134 and CD137 expression. Long term studies, enrolling larger groups of patients, including their subdivision to colon and rectal cancer, are required to utilize CD134 and CD137 in immune therapy of colorectal cancer.

**Key words:** adhesion activity, adhesion molecules, CD134, CD137, colorectal cancer

Epidemiological studies prove that incidence of colorectal cancer is increasing. This trend is particularly clear in highly developed countries where malignancies of this segment of the gastrointestinal tract are the second most common (after lung cancer) cause of death due to malignancies. The first line therapy of colorectal cancer is surgical resection of the primary tumor and elimination of regional and remote metastases. Often combination therapy is required to achieve complete cure or significant slowing of the malignant process (1-4).

Until recently, main interest of the surgeons were focused on new diagnostic techniques, surgical techniques and conventional adjuvant treatment methods (chemo – and radiotherapy) (4). Recently, due to a significant progress in molecular biology, searching for genes responsible for cellular mutations and development of cancer became the main research direction (1, 5, 6). Molecular therapies for the treatment of the colorectal cancer are still under development. Effect of specific macromolecules (DNA, antibodies, viruses) on cancer metabolism is still being investigated (1, 5, 6, 7).

The biggest challenge for the researchers is modification of the treatment procedures for metastatic patients, in whom metastases re-
fect advancement of the disease process. In such cases modern methods of immune therapy may improve prognosis after the surgical procedure. Immune defense mechanisms are switched when tumor cells develop, i.e. when natural anti-cancer defenses of the body become insufficient. Many authors indicate crucial role of immune system in anti-cancer protection (1, 5, 8, 9, 10).

Carcinogenic antigens are studies to determine their utility in the detection, monitoring and treatment. Monoclonal antibodies to cancer antigens may be an example (2, 6, 7, 8, 11). Recently also attempts have been made to use various vaccines to stimulate immune response, in particular cell-mediated immune response (12). Therefore the most recent studies of locoregional expression and relation between formation of colorectal cancer metastases are aimed at defining defense mechanisms and forms of immune therapy. Modern methods of cancer therapy are aimed at modification of immune processes so that they can be used as adjunctive methods to conventional therapeutic procedures.

Adhesion molecules are a large group of molecules of diverse structure and function. They are involved in the immune response in multiple diseases. The most commonly studies molecules include: CD49d, CD54, CD11a, CD44, CD102, CD62L. Recently, role of receptors CD134 (OX-40) and CD137 (4-1BB) Has been emphasized in the progression and therapy of colorectal cancer (3, 5, 13-18).

The CD134 (OX-40) receptor and OX-40L ligand belong to TNFR superfamily. When contacted with an antigen, CD134 produces interleukin IL-4 and other cytokines. Activated OX-40 also results in increased production of antibodies by B lymphocytes. It is expressed on activated T lymphocytes, B lymphocytes and on dendritic and endothelial cells. Interleukin IL-4 induces IgE production by B lymphocytes and simultaneously stimulates monocytes and macrophages that become more cytotoxic against the tumor cells (phagocytosis intensification) (13, 14, 17).

CD137 (4-1BB) molecule is present on the surface of T lymphocytes and NK cells (natural killer). A ligand for this molecule is present on B cells, macrophages and dendritic cells, where it is expressed. Such co-stimulation results in increased production of interleukin IL-2 by T lymphocytes that stimulates proliferation of T lymphocytes, NK cells and cytotoxic lymphocytes (1, 5, 14-17).

Determination of CD134 and CD137 levels at various stages of malignancy and at various times may be very helpful in the analysis of the treatment results as well as can be used to determine prognosis, risk of local and remote recurrence. Its most important benefit seems to be related to a chance of development of an adjunctive treatment model using immune therapy.

The aim of this study was to determine expression of adhesion molecules CD134 and CD137 in the peripheral blood of colorectal cancer patients, depending on stage of the tumor, its size and invasion.

MATERIAL AND METHODS

The study enrolled 72 patients with primary colorectal adenocarcinoma – 43 men and 29 women. Age range of the patients was from 32 to 86 years (average 64.55 years). All patients underwent surgical treatment at 2nd Department of General and Gastroenterological Surgery, Medical University of Białystok.

Clinical stage of the tumor was assessed using Dukes scale: A – 6 patients (8.32%), 3 men and 3 women, B – 34 (47.22%) 20 men and 14 women, C – 20 (27.79%) 12 men and 8 women, D – 12 (16.67%) – 8 men and 4 women. According to Astler and Coller scale: A – 6 patients (8.32%), B1 – 15 (20.8%), B2 – 19 (26.4%), C1 – 10 (13.9%), C2 – 10 (13.9%), D – 12 (16.67%).

Expression of adhesion molecules was assessed in the venous peripheral blood collected on the day of the procedure and 10 days after the procedure (such schedule was related to the protocol of animal experiments). Lymphocytes were isolated using a mechanical isolation method. They were washed twice and counted in the Burker chamber. Then a suspension of 10^6 nuclear cells /ml RPU/1640 was prepared. To prepare another 100 µl samples, 10 µl of each of monoclonal antibodies CD134 and CD137 were added from the ready kits: CD134 (OX-40) – FITC – 34464 X and CD137 (4-1BB) – PE – 34465 X (Pharmin-gen). After 20 minutes of incubation at room temperature, the samples were carefully mixed and analyzed quantitatively in a flow cytometer EPICS XL (Coulter); each time 10^4 cells were analyzed.
Obtained results were subjected to statistical analysis using Fisher’s test. p<0.05 was considered statistically significant.

RESULTS

Figure 1 demonstrates analysis of mean CD134 and CD137 expression. Mean activity of CD134 (12.66%) was significantly higher than that of CD137 (6.26%) (p<0.001). Similar differences were observed both on day 1 and day 10 after the surgical procedure. Additionally, a significant increase of activity of both these adhesion molecules was observed within 10 days after the surgical procedure (CD134 from 10.07 to 15.25; CD137 from 4.91 to 7.61) (p<0.01).

Stage on Dukes scale, according to our assessment, had no effect on CD134 activity (fig. 2); such results were found both in samples collected on the day of the procedure as well as 10 days after the procedure.

However, relationship between CD137 activity and tumor stage was different (fig. 3). No differences were found in CD137 expression for individual colorectal cancer stages on the day of the procedure. On day 10 after the procedure, increased CD137 activity was observed in individual colorectal cancer stages (Dukes A – 2.62%, B – 5.18%, C – 8.16%, D – 14.48%) (p<0.001).

CD134 expression did not change any of the studies for any stages of invasion of the tumor (fig. 4).

Figure 5 presents relation between CD137 activity and depth of the tumor invasion. CD137 expression did not change for any stages in tests performed on day 1 or day 10 after the procedure. However, a significant reduction of CD137 activity along with increased tumor invasion was observed (pT1 – 9.53% – 8.12%; pT4 – 1.95% – 2.43%).

DISCUSSION

Malignancy is initiated at various levels. Carcinogens act through indirect activation, leading to tumor formation. DNA damage results when immune system has not been activated yet or when activity of anticarcinogenic factors is not sufficient. At this time immune mechanisms start their activity (2, 7).

Difficulties related to use of tumor antigens in diagnosis, monitoring and treatment of
malignancies results from the fact that majority of them are nonspecific.

Modification of activity of immune system in the treatment of malignancies may supplement other methods of combination treatment. Literature reports two classifications of immune therapy. First divides methods into active (stimulation of immune reactivity of the patient), passive (use of antibodies) and adoptive (application of cells of immune system). The second classification includes specific (vaccines, antibodies, lymphocytes) and nonspecific methods (immunostimulation, cytokines). Selection of a treatment method requires knowledge of effects of the disease on the immune system and is possible provided that precise level of immune parameters is established for the health and disease. It is essential for establishment of standards of immunotherapeutic management (6, 8, 10, 12).

Assessments of expression of adhesion molecules CD134 and CD137 available in the literature, were done mainly in animal models (1, 5, 8, 9, 11, 16); only few of them were done in humans (3, 15, 17).

We determined CD134 and CD137 activity in human tissues. We found a significant increase of CD134 and CD137 expression over 10 days after the surgical procedure. These results modify our previous conclusions where we found an opposite relation in a smaller group of patients (17). The current results suggest increased interleukin production after elimination of a tumor. It also suggests increased production of IgE, NK and cytotoxic lymphocytes.

Our studies indicate increased CD134 and CD137 expression with higher tumor stage. Depth of invasion had no effect on CD134 activity, but increased CD137 expression was evident. Such correlation was observed both on the day of the procedure and on day 10 after the procedure. No such correlations were found in the available literature. However, results of activity of the studied molecules in the tumor mass and adjacent tissues were reported. High expression of CD134 correlated with better patient survival (3, 13). This suggests that CD134 could be used in the immune therapy of the colorectal cancer.

CD137 assessments done in animal models suggested possible use of CD137 molecules in the treatment of the colorectal cancer with hepatic metastases. Limitation of this method is lack of assessments of CD137 activity in humans (1, 5).

Some researchers reported use of immune therapy in some tumors, including colorectal cancer (18). Two molecules assessed by us could be used to guide treatment and prognosis in the colorectal cancer. CD134 expression could have effect on prophylaxis of metastases, while CD137 on the treatment of patients with metastases (mainly to the liver) which was supported by results of our study.

Our study enrolled patients with the colorectal cancer. However, it must be emphasized that Dimbert et al. found markedly higher CD137 expression in patients with the rectal cancer than in subjects treated for colon cancer. Therefore it seems justified to study separately patients with colon and rectal cancer (15).

Determination of CD134 and CD137 activity in a larger group of patients could result in new possibilities of treatment and prognosis in the colorectal cancer. Detailed interpretation of these results could properly guide assessment of risk of metastases and their prophylaxis. Subsequently it could even lead to development of treatment options of liver metastases of the colorectal cancer.

CONCLUSIONS

1. CD137 activity is directly proportional to stage of the colorectal cancer.
2. Surgical resection of the tumor results in increased CD134 and CD137 expression.
3. For CD134 and CD137 to be used in immune therapy of the colorectal cancer, long term studies in larger groups of patients must be conducted, including sub-division of patients with rectal and colon cancer.

REFERENCES

of the T-cell costimulatory molecule OX-40 (CD134).


