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ORIGINAL ARTICLE

The Dysfunction of NK Cells in Patients with Type 2 Diabetes and Colon Cancer

Paweł Piątkiewicz · Tomasz Miłek · Małgorzata Bernat-Karpińska · Monika Ohams · Anna Czech · Piotr Ciostek

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Abstract Glucose metabolism disorders influence anti-carcinogenic function of natural killer (NK) cells. The aim of this study was to evaluate the number and cytotoxic activity of NK cells in type 2 diabetic (T2D) patients with negative family history of cancer, type 2 diabetic subjects with newly diagnosed untreated colon cancer (T2DCC) and patients without type 2 diabetes with newly diagnosed, untreated colon cancer (CC). Incubation tests were performed in 18 T2D patients, treated with diet and oral antidiabetic agents, 16 T2DCC; cT1-4N0M0 (c-clinical diagnosis based on computed tomography, colonoscopy and histopathology) treated with diet and oral antidiabetic agents and 16 normoglycemic CC; cT1-4N0M0. Control group included 18 metabolically healthy (with normal fasting glucose and normal glucose tolerance) subjects (HS) with negative family history of cancer, matched by age, BMI and waist circumference. Peripheral blood mononuclear cells were isolated by means of gradient centrifugation. The K562 human erythroleukemia cell line served as the standard target for human NK cytotoxicity assay. The T2D revealed an increased number of NK cells (13.56 ± 5.9 vs 9.50 ± 4.8 %; $p < 0.05$) when compared with HS, yet these cells had a decreased activity (3.3 ± 2.5 vs 9.4 ± 3.6 %; $p < 0.01$). The CC demonstrated a decreased activity (2.9 ± 1.8 %; $p < 0.01$) but a similar number (8.82 ± 3.7 %; not significant) of NK cells when compared to HS. The T2DCC NK cells were characterized by trace cytotoxic activity (1.1 ± 0.7 %; $p < 0.01$) and nearly three times greater amount (21.24 ± 7.5 %; $p < 0.01$) when compared to T2D. Type 2 diabetes and CC are associated with disadvantageous alterations of NK cells, leading to impairment in their cytotoxic activity. The impaired activity of NK cells in T2D can be involved in the increased carcinogenic risk and can promote a higher incidence of CC.

Keywords NK cells · Diabetes · Colon cancer

P. Piątkiewicz (✉) · M. Bernat-Karpińska · A. Czech
Chair and Department of Internal Diseases and Diabetology, Medical University of Warsaw, Warsaw, Poland
e-mail: piatkiewicz@op.pl

T. Miłek · P. Ciostek
Chair and Department of General and Vascular Surgery, Medical University of Warsaw, Warsaw, Poland

M. Ohams
Department of Clinical Immunology, Medical University of Warsaw, Warsaw, Poland

Introduction

Natural killer (NK) cells constitute from 5 to 19 % of the peripheral blood lymphocytes, and most of them have morphology of a large granular lymphocyte (Hannet et al. 1992; Timonen et al. 1981). They are characterized by the ability to destroy target cells spontaneously, without prior immunization, and their cytotoxic effect is not subject to any major histocompatibility complex (MHC) restrictions (Lanier et al. 1986; Ritz et al. 1988; Trinchieri 1989).

Natural killer cells were first described during the study of specific lymphocyte cytotoxicity against neoplastic cells (Herberman et al. 1975a, 1975b; Rosenberg et al. 1972). Subsequent studies showed that the cells responsible for cytotoxic activity are devoid of cell surface antigens, characteristic of B and T lymphocytes (Herberman and Ortaldo 1981; Ozer et al. 1979). The use of monoclonal antibodies and molecular biology techniques enabled to

identify NK cells as a distinct population of cells exhibiting natural cytotoxicity (Robertson and Ritz 1990).

Natural killer cells originate from precursors maturing in the bone marrow. In bone marrow transplant recipients treated because of hematological malignancies, the appearance of NK cells in blood with an NK cell phenotype of the donor was observed (Hercend et al. 1986; Rooney et al. 1986). Moreover, the phenotype of lymphocytes and NK cell activity can also be obtained in vitro from bone marrow precursor cells (Keever et al. 1989; Migliorati et al. 1989; Van den Brink et al. 1990).

In patients with diseases such as Chediak–Higashi syndrome or the X-linked lymphoproliferative syndrome, a slight NK cell activity and increased incidence of cancer were observed (Pross 1986; Roder et al. 1980; Sullivan et al. 1980). It was shown that the lower NK cell activity is associated with a higher risk of developing cancer. The NK cell activity in cancer patients decreased with increasing severity of the cancer (Held et al. 2011).

In the process of destroying neoplastic cells, NK cells most likely play a complementary role for T lymphocyte cells. They destroy neoplastic cells that are not capable of presenting antigen into T lymphocytes and cannot be recognized by them or eliminated. These cells are characterized by low expression of MHC class I molecules or a complete lack of these molecules (Moretta and Moretta 2004; Moretta et al. 2005).

Impaired NK cell activity may contribute to an increased susceptibility to cancer in patients with diabetes. Significant differences in the number and activity of NK cells, which have been shown in patients with type 2 diabetes (T2D) in comparison to metabolically healthy subjects (HS) (Spooren et al. 1993), may contribute to a better understanding of the pathomechanism and more frequent incidence of neoplasms in subjects with T2D.

It has been shown that colon cancer (CC) is a neoplasm occurring quite frequently in the population of patients with T2D compared to people without diabetes (Berster and Göke 2008; Gallagher and LeRoith 2011; Hjartåker et al. 2008). It is further considered that hyperglycemia, hyperinsulinemia and insulin resistance are independent risk factors relating this neoplasm (LeRoith et al. 2008). Identification and characterization of precise circumstances underlying the occurrence of CC in people with T2D are potentially important for basic studies focusing on the biology of cancer. It is likely that the results and conclusions from this study will also be applicable in the field of clinical oncology. This problem should be considered a priority due to the high and steadily increasing incidence of both CC and diabetes, making these conditions the core issue of prevention programs for the population.

Allowedly, in people with T2D there is a higher occurrence of CC (Tseng 2012). The aim of this study was to verify the hypothesis that the above relationship can be associated with dysfunction of NK cells.

The specific objective of this study was to demonstrate the differences in the number and cytotoxicity of NK cells in patients with T2D and active cancer (CC) compared to patients with T2D without this cancer.

Materials and Methods

Four groups have been created to realize the objectives of the study:

(A) individuals with CC in stage T1-4N0M0 and T2D treated with diet and/or oral antidiabetic agents;
(B) patients with CC in stage T1-4N0M0 with normal glucose tolerance;
(C) patients with T2D treated with diet and/or oral antidiabetic agents without the above-mentioned cancer and with a negative history of cancer;
(D) healthy individuals with normal glucose tolerance and with a negative history of cancer, who constituted the control group.

All people participating in the study were informed in detail about the conducted experiments and the safety principles before they were allowed to give their consent to participate in the study. Qualifying tests were conducted under the protocol approved by the Commission of Bioethics at the Medical University of Warsaw (No. 189/2009 of 20 October 2009).

Recruitment and qualification of all the research groups were performed at the Department of Internal Medicine and Diabetology, and the First Chair and Clinic of General and Vascular Surgery Second Faculty of Medicine, Medical University of Warsaw. Patients were divided into different groups based on general internal examination, with particular emphasis on family interviews (standardized questionnaire survey to collect information including demographic, environmental and clinical data) and biochemical tests.

Biochemical tests were performed (in groups A and B before surgery) in the scientific laboratory of Department of Internal Medicine and Diabetology and included the determination of:

- fasting blood glucose: with enzymatic method using BIOSEN 5040 analyzer (EKF-Diagnostic GmbH, Germany);
- glycated hemoglobin (HbA1c): with HPLC method using Variant analyzer (Bio-Rad Laboratories Inc, USA);
- insulin: with radioimmunoassay IMMULITE 2000 Insulin (Siemens Healthcare Diagnostics, USA);
- HOMA-IR index [HOMA-IR = fasting insulin (mU/l) × fasting glucose (mmol/l)/22.5];

- oral glucose tolerance test: 75 g of glucose according to WHO procedures (in groups B and D).

The study excluded people:

i. with renal insufficiency (serum creatinine >2.5 mg/dl);
ii. with liver diseases (transaminases exceeding the upper reference value >3 times);
iii. with symptomatic heart failure;
iv. with diabetic complications;
v. using diabetogenic drugs (e.g. systemic corticosteroids, oral contraceptives, thiazide diuretics, miconazole);
vi. abusing drugs, alcohol, dependent on drugs during the last year;
vii. pregnant women, women during lactation.

NK Cytotoxicity Assay

Separation of Effector Cells

Peripheral blood mononuclear cells were isolated from heparinized blood of patients by Ficoll gradient centrifugation (Aqua-medica, Krakow). Mononuclear cells were adjusted to 1×10^7/2.6 ml in culture medium (RPMI 10 %FCS) (RPMI1640—FCS: fetal calf serum; Sigma-Aldrich).

Preparation of Target Cells

The K562 human erythroleukemia cell line was used as the standard target (T) for human NK assay. K562 cells were washed in PBS and labeled with green dye, DIO (3,3-dioctadecyloxacarbocyanine perchlorate; Sigma-Aldrich) for 20 min at 37 °C, 5 % CO_2. Target cells were washed twice in PBS and adjusted to 10^6 cell/ml in medium (RPMI 10 % FCS) (Czech et al. 2009).

Evaluation of Cytotoxicity Assay

Target and effector cells were added to reach effectors'/targets' ratios: 50:1, 12:1. Dead target cells were stained with red dye, propidium iodide, (Sigma-Aldrich). After 4 h of incubation at 37 °C, 5 % CO_2, samples were collected for analysis on the Becton–Dickinson FACSCalibur Flow Cytometer using the Cellquest Program.

Live target cells (T) were identified with strong green fluorescence, whereas dead target cells (Td) showed strong green and red fluorescence.

The percentage of dead target cells (Td %) was calculated as follows:

%Td = (Td/T) × 100 %. Specific lysis was calculated as %Td (cultured with effectors cells) − Td (cultured without effectors cells).

Phenotyping NK Cells

Immunofluorescent phenotyping of NK ($CD16^+$) and cytotoxic T cells ($CD3^+$, $CD56^+$) in peripheral blood lymphocytes was performed using specific murine anti-human CD3/$CD56^+$CD16 FITC-PE-conjugated monoclonal antibodies (Simultest, Becton–Dickinson). Fluorescence analysis was performed using FACSCalibur flow cytometer and the Cellquest program. Lymphocytes T ($CD3^+$) cells were identified with strong green fluorescence, NK cells ($CD16^+$) with red fluorescence and cytotoxic lymphocytes ($CD3^+CD56^+$) were identified with green and red fluorescence (Johann et al. 1995; Kane et al. 1996).

These studies were conducted at the Department of Clinical Immunology at the Transplantation Institute, Medical University of Warsaw.

The diagnosis of CC was established based on the medical history, as well as physical, endoscopic and histopathological examination. Computed tomography of the abdomen was also performed to determine the staging of cancer (cTNM). The study included patients with clinical diagnosis of CC without lymph node metastases and distant metastases (T1-4N0M0). The operation was conducted within a period of up to 14 days after establishing the clinical diagnosis at the First Chair and Clinic of General and Vascular Surgery, Second Faculty of Medicine, Medical University of Warsaw. Histopathological results verified the clinical diagnosis. In all analyzed cases, the presence of adenocarcinomas was registered. Due to the detection of metastasis in mesenteric lymph nodes, four patients were excluded from the group of 20 people with CC and three patients from the group of 19 patients with T2D and CC.

Finally, the analysis included a total of 68 subjects:

- 16 patients with CC in stage T1-4N0M0 and T2D treated with diet and/or oral antidiabetic agents;
- 16 patients with CC in stage T1-4N0M0 with normal glucose tolerance;
- 18 people with T2D treated with diet and/or oral antidiabetic agents without the above-mentioned cancer and with a negative history of cancer;
- 18 HS with normal glucose tolerance and with a negative history of cancer, which constituted the control group.

Statistical Methods

These data were presented as a mean and standard deviation. Quantitative parameters were compared using the Aspin-Welch test with a marginal level of statistical significance equaling $p = 0.01$. This test is the optimal statistical method for such calculations, because it does not

Table 1 Characteristics of the study groups

Parameter ($x \pm$ SD)	Control group	Type 2 diabetes (T2D)	Colon cancer (CC)	T2D CC
Number of patients	18	18	16	16
Sex (M/F)	8/10	9/9	7/9	8/8
Age (years)	52.4 ± 7.0	53.6 ± 7.0 ns	55.2 ± 8.0 ns	55.0 ± 8.0 ns
BMI (kg/m^2)	26.3 ± 3.0	26.7 ± 5.1 ns	25.7 ± 3.0 ns	26.1 ± 3.0 ns
WHR	0.87 ± 0.09	0.88 ± 0.08 ns	0.87 ± 0.09 ns	0.88 ± 0.09 ns
Duration of diabetes (years)	–	6.3 ± 2.9	–	6.5 ± 3.0 NS

Comparison of the parameters of various study groups was made relative to the control group

ns not significant; *NS* not significant in relation to T2D group; *BMI* body mass index, *WHR* waist/hip ratio

require equal variance from the breakdown of two random variables. Multiple factor regression was performed to analyze correlations of NK cells number and activity with all studied clinical parameters (Armitage 1971).

Results

The characteristics of the studied groups are shown in Table 1. The type of antidiabetic therapy in patients with T2D is shown in Table 2. Laboratory results obtained in different groups of patients are shown in Table 3.

The studied groups did not differ in a statistically significant manner in terms of numbers, gender, age, BMI and WHR. Only the duration of diabetes in patients with T2D was similar (statistically insignificant) to its duration in patients with both T2D and CC (Table 1).

Subjects with T2D and CC differed significantly from those with T2D without cancer in terms of diabetic therapy. Metformin was the predominant antidiabetic drug in patients with diabetes and without cancer, as it was used in 83.3 % of patients. The vast majority of people with T2D and CC (75 %) was treated with a sulfonylurea (Table 2).

The groups of people with T2D and the group of people with both T2D and CC were characterized by higher mean values of biochemical parameters evaluated in comparison with the control group. In patients with only T2D a significantly higher level of fasting plasma glucose (7.20 vs 5.37 mmol/l, $p < 0.01$) and HbA1c (7.13 vs 5.4 %; $p < 0.01$) was noticed in comparison to the control group, as well as significantly higher levels of insulin resistance. The mean fasting insulin and HOMA-IR index in this group amounted to 14.04 vs 7.89 mU/l ($p < 0.01$) and 4.47 vs 1.88 ($p < 0.01$). In patients with both T2D and CC, the mean fasting plasma glucose (7.45 vs 5.37 mmol/l; $p < 0.01$) and HbA1c (7.17 vs 5.4 %, $p < 0.01$) were significantly higher compared to the control group, but did not differ significantly with respect to a group of people with only T2D. In addition, this group (CC + T2D) was characterized by the highest mean fasting insulin (20.91 mU/l) and the highest value of the insulin resistance index: HOMA-IR (6.92) among all studied groups, and the resulting differences were statistically significant ($p < 0.01$). In patients with CC and normal glucose tolerance, the mean values of the tested biochemical parameters were significantly lower than in patients with T2D and also in the group of people with both T2D and CC. This group did not show statistically significant differences relating the control group (Table 3).

Table 2 The type of antidiabetic therapy in patients with type 2 diabetes (T2D) and in patients with both colon cancer and type 2 diabetes (T2DCC)

Antidiabetic drug	T2D number of cases (%)	T2DCC number of cases (%)
Metformin	15/18 (83.3)	8/16 (50)
Sulfonylurea	4/18 (22.2)	12/16 (75)
Acarbose	3/18 (16.7)	2/16 (12.5)
Incretin mimetics	2/18 (11.1)	0/16 (0)

Subjects with T2D showed a significantly increased number of NK cells in comparison to the healthy control group (14.21 ± 9.52 vs 5.9 ± 4.8 %; $p < 0.01$). Cytotoxic activity of NK cells of patients with T2D was significantly reduced compared to the healthy control group (4.0 ± 2.5 vs 9.4 ± 3.6 %; $p < 0.01$). NK cells of people with both T2D and CC were characterized by a trace amount of cytotoxic activity (1.1 ± 0.7 vs 9.4 ± 3.6 %; $p < 0.01$), and their number was significantly higher in comparison with the control group (20.06 ± 6.8 vs 4.8 ± 9.52 %; $p < 0.01$). In patients with CC without carbohydrate metabolism disturbances, a significant reduction of cytotoxic activity (2.9 ± 1.8 %; $p < 0.01$) (Fig. 1) in relation to the control group with an unchanged number of NK cells (9.18 ± 3.7 %; not significant) was noted (Fig. 2).

People with T2D and coexisting CC were characterized by a significantly higher number (20.06 ± 6.8 %) and significantly lower activity of NK cells (1.1 ± 0.7 %) compared to those with T2D and without cancer ($p < 0.01$), as well as with individuals with CC and normal glucose tolerance ($p < 0.01$). In people with T2D, a higher number

Table 3 Comparison of laboratory tests results

Parameter ($x \pm$ SD)	Control group	T2D	CC	T2DCC
Fasting plasma glucose (mmol/l)	5.37 ± 0.68	7.20 ± 0.67 $p < 0.01$	5.46 ± 0.74 ns	7.45 ± 0.95 $p < 0.01$
HbA1c (%)	5.4 ± 0.7	7.13 ± 0.61 $p < 0.01$	5.45 ± 0.61 ns	7.17 ± 0.93 $p < 0.01$
Fasting insulin (mU/l)	7.89 ± 3.65	14.04 ± 1.59 $p < 0.01$	8.12 ± 4.63 ns	20.91 ± 8.09 $p < 0.01$
HOMA-IR[a]	1.88 ± 1.03	4.47 ± 1.98 $p < 0.01$	1.97 ± 1.1 ns	6.92 ± 2.8 $p < 0.01$

Comparison of the parameters of various study groups was made relative to the control group

ns not significant

[a] HOMA-IR index was calculated based on the HOMA formula = fasting insulin (mU/l) × fasting glucose (mmol/l)/22.5

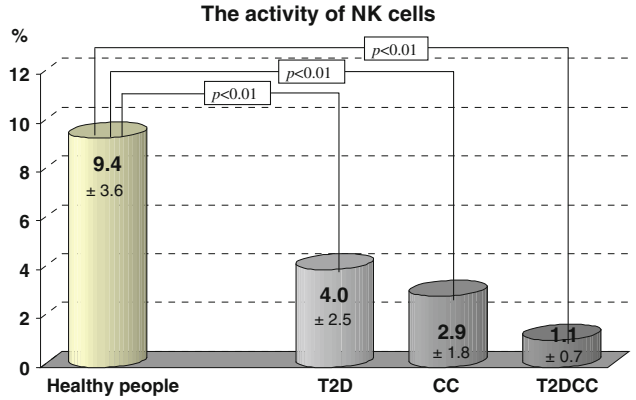

Fig. 1 NK cell activity in the studied groups

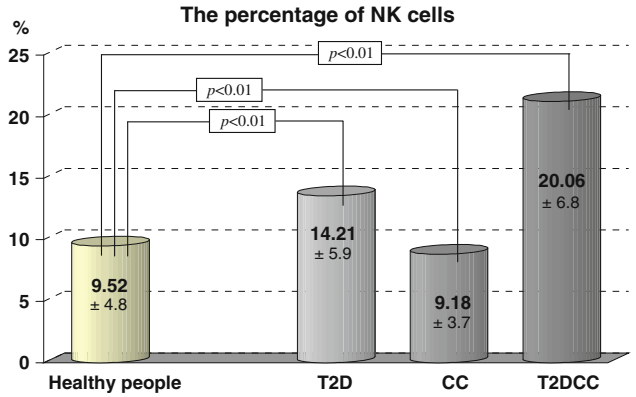

Fig. 2 The percentage of NK cells in the studied groups

of NK cells was observed when compared with the group of people with CC was noted (14.21 ± 9.18 vs 5.9 ± 3.7 %; $p < 0.01$). However, there was no statistically significant difference in the cytotoxic activity of NK cells in these patients (4.0 ± 2.5 vs 2.9 ± 1.8 %; not significant) (Figs. 1, 2).

We have found no simple correlations between NK cells activity and other clinical factors in the studied groups. The only exception was the group of patients with T2D and CC, where we have found a medium positive correlation between NK cells activity and age (Table 4). Multiple factor regression performed for all studied groups did not reveal any significant factors influencing NK cells number. Statistical analyses revealed that the NK cells activity has not been significantly influenced by any clinical factors in the control group, T2D group and CC group. Multiple factor regression performed for the group with both T2D and CC showed that the following factors have a significant influence on NK cells activity: age ($p < 0.001$), HbA1C ($p < 0.0002$), fasting plasma insulin ($p < 0.0075$; Table 5).

Table 4 The correlations between NK cells activity and other clinical factors in T2DCC group

Variable	Pearson correlation	p
NK cells activity and age	0.56	0.02[a]
NK cells activity and height	−0.20	0.47
NK cells activity and weight	−0.34	0.20
NK cells activity and BMI	0.47	0.06
NK cells activity and waist	0.01	0.97
NK cells activity and hip	0.01	0.97
NK cells activity and WHR	0.12	0.65
NK cells activity and SBP	−0.08	0.76
NK cells activity and DBP	−0.30	0.25
NK cells activity and FPG (mg/dl)	0.36	0.18
NK cells activity and T2D duration (years)	−0.03	0.90
NK cells activity and HbA1c	0.41	0.11
NK cells activity and insulin	0.14	0.61
NK cells activity and C-peptide	0.19	0.48
NK cells activity and HOMA	0.14	0.61

SBP systolic blood pressure; *DBP* diastolic blood pressure; *FPG* fasting plasma glucose

[a] Medium positive correlation

Discussion

Proper identification of people with T2D with additionally an increased risk of cancer is essential to implement adequate preventing actions. Given the alarming epidemiological data on the prevalence of both T2D and CC (Piątkiewicz and

Table 5 Multiple factor regression of clinical factors influencing NK cells activity in T2DCC group

Source	DF	Sum of squares	Mean square	*F* value	$p > F$
Model	4	0.16607	0.04152	16.53	0.0001
Error	11	0.02763	0.00251		
Corrected total	15	0.1937			
Variable	**Parameter estimate**	**Standard error**	**Type II SS (types of sums of squares)**	***F* value**	$p > F$
Intercept	−1.64049	0.55098	0.02227	8.86	0.0126
Age	0.01926	0.00284	0.11556	46	0.001
HbA1c	0.30948	0.05748	0.07283	28.99	0.0002
Insulin	0.01857	0.00568	0.02685	10.69	0.0075

Multiple factor regression for the T2DCC group showed that the following clinical factors have a significant influence on NK cells activity: age $p < 0.001$, HbA1c $p < 0.0002$, insulin $p < 0.0075$

DF degrees of freedom; *F value* Fisher's test

Czech 2011), an effective action within the scope of primary prevention would not only lead to individual benefits in the large number of patients, but will also have spectacular economic and social benefits. For over 20 years, research programs have been conducting tests for CC screening, and currently full colonoscopy remains to be the preferred screening method in Poland and the developed countries of the world (Burt et al. 2010; Rex et al. 2009).

According to current guidelines, the Polish Society of Gastroenterology recommends performing it once every 10 years in people over 50 years of age and in people with an increased risk of this cancer (positive family history, genetic factors), and these studies are repeated frequently and initiated earlier. Unfortunately, none of gastroenterological scientific societies regards patients with T2D as those with a high risk of developing CC. Moreover, the Polish Diabetes Association guidelines for the year 2011 do not provide any information on the need for greater vigilance in patients with T2D despite the fact that large clinical trials have shown that T2D is associated with a significantly increased risk of CC (Atchison et al. 2011; Flood et al. 2010; Hemminki et al. 2010; Inoue et al. 2006; Larsson et al. 2005).

A clear decline in the incidence and mortality as a result of CC among people who underwent colonoscopy screening tests was exhibited (Kahi et al. 2009), but in many cases the diagnosis is established relatively late, which is largely due to large time intervals between successive tests. For years, there has been a discussion concerning the best screening methods in the diagnosis of this cancer. The efforts of researchers focused on understanding of pathogenetic mechanisms, which present the link between diabetes and CC. Proper understanding of these mechanisms could lead to future development of effective diagnostic tests for assessing the risk of CC in patients with T2D.

It is believed that embryonic level of NK cell activity results from congenital (hereditary) and acquired (environmental) factors. This level is probably determined during the first year of life and is a subject to slight fluctuations depending on the impact of the environment, although it does not undergo significant changes throughout lifetime (Piątkiewicz et al. 2010).

The available literature provides theses concerning the number and function disorders of cells in the immune system cells in people with diabetes. The relation between the increased cytotoxic activity of NK cells and the pathogenesis of this disease is explained (Piątkiewicz et al. 2010). However, some believe that these disorders which arise secondary to diabetes and simply are consequences of diabetes. It has been shown that disturbances in the functioning of immune cells, including NK cells, are very similar both in type 1 and T2D (Spooren et al. 1993).

It is believed that the decreased NK cell activity is associated with an increased risk of developing cancer. It was found that in patients with limited metastatic process, NK cell activity is comparable to the activity of NK cells in healthy people or is only slightly smaller, while in patients with advanced cancer, this activity decreases significantly. It is suggested that the reduction of NK cell activity in these patients is rather an effect, and not a cause of cancer (LeRoith et al. 2008).

This study was focused on determining the impact of T2D and CC on the number and cytotoxic activity of NK cells. These cells were isolated from the peripheral blood of people with T2D without cancer, from people with CC and a normal glucose tolerance, and from people who exhibited the co-existence of both pathologies. Our study

showed that the number of NK cells increased in subjects with T2D without cancer as well as in subjects with co-existing T2D and CC when compared to HS. Simultaneously, we observed no statistically significant difference concerning the number of NK cells in patients with CC and normal glucose tolerance in comparison to the control group.

Natural killer cell activity in all the groups was significantly reduced when compared with HS. No statistically significant differences between NK cell activity in patients with T2D without cancer and those with CC with normal glucose tolerance were registered. We can conclude that a reduced cytotoxic activity of NK cells was observed during the course of both T2D and CC was observed. It should therefore be noted that the impairment of NK cell activity is particularly prominent when both diseases co-exist.

It is worth noting that the observed impairment relating cytotoxic activity of NK cells in all groups was not related to the level of glycated hemoglobin HbA1c, and this could indicate that inadequate metabolic control is not the only cause of immune disorders, which in turn led to impaired immunity and increased susceptibility to cancer in T2D. Some researchers suggest that defects in functioning of the immune system are associated with abnormal metabolic control of diabetes (Moutschen et al. 1992).

In this study, changes in number, especially in the cytotoxic activity of NK cells in the group of people with T2D and a co-existing CC were accompanied by increased levels of fasting insulin and high HOMA-IR index value. We can therefore conclude that there is a correlation between the abnormal number of NK cells and their reduced cytotoxic activity, as well as the state of insulin resistance. These changes were observed in people with T2D whose impaired immune system leads to a significant impairment in antitumor defense, and is probably related to metabolic disorders, particularly hyperinsulinemia.

It should be noted that the results obtained from the majority of prospective clinical trials in various populations have indicated a positive and statistically significant correlation between high levels of endogenous insulin in plasma and an increased risk of cancer or increased mortality due to cancer, however, these results vary depending on the type of cancer and the studied population (Piątkiewicz and Czech 2011).

During a prospective study carried out by European Prospective Investigation into Cancer and Nutrition (EPIC), it has been shown that hyperinsulinemia expressed by elevated C-peptide levels positively correlated with an increased risk of CC (Jenab et al. 2007). Similar results were obtained in the study of a population of nurses (Nurses' Health Study). High C-peptide levels correlated with the risk of CC and rectal cancer [relative risk 1.76 (95 % CI: 0.85–3.63)]. No influence on the level of HbA1c towards the risk for cancer was noted (Wei et al. 2005).

However, our study revealed that the impairment in the cytotoxic activity of NK cells in the group of patients with T2D and co-existing CC correlated with increased levels of both fasting plasma insulin ($p < 0.007$) and HbA1C ($p < 0.0002$).

In a slightly earlier study of a female population in New York, the effect of C-peptide concentration levels in contribution to the risk of colon and rectal cancer was assessed. The risk of cancer rose together with increasing C-peptide concentration (OR: 2.92; 95 % CI: 1.26–6.75) (Kaaks et al. 2000). The same dependence has been demonstrated in males. In a prospective population study of doctors (Physicians' Health Study), a positive correlation between C-peptide concentrations and CC risk has also been demonstrated (top vs bottom quintile RR: 2.7, 95 % CI: 1.2–6.2) (Ma et al. 2004).

In our study, subjects with T2D without cancer differed from those with T2D and CC in terms of applied antidiabetic therapy. Metformin was predominantly used in patients with T2D and without cancer, while the vast majority of people with T2D and coexisting CC was treated with a sulfonylurea derivative.

Epidemiological studies consistently show that metformin intake reduces the risk of CC (Currie et al. 2009; Libby et al. 2009; Tseng 2012). The protective effect of metformin appears to result from its inhibitory effect on intracellular transduction mechanisms, among others, such as the ones stimulated by the activation of insulin receptor axis/IGF (Buzzai et al. 2007; Feng et al. 2011; Gonzalez-Angulo and Meric-Bernstam 2010) among others. On the contrary, this method of treating diabetes, which is associated directly with an increasing insulin levels, both exogenous and endogenous increases the risk of cancer.

Therefore, it can be emphasized that patients with T2D face an increased risk of developing cancer, and in the case of its occurrence, a worse prognosis. Thus, patients with T2D are at increased risk of developing cancer, such as CC and should be subject to enhanced diagnosis, as far as the above is concerned. You should also remember that the risk of cancer and its course may influence the selection of the appropriate treatment for diabetes (Piątkiewicz and Czech 2011).

To conclude with, we can hypothesize that the impaired NK cell activity probably contributes to increased susceptibility to CC in patients with T2D. We showed that significant differences in NK cell activity, in people with cancer and T2D compared to metabolically HS, can lead to a better understanding of the pathophysiological mechanism underlying the frequent incidence of CC in people with T2D. Restoring normal functions of NK cells may become one of the goals associated with hypoglycemic

therapy, particularly in terms of enhancing antitumor protection. Our study reveals that the above-mentioned compensation mechanism is a clear failure in the case of cancer, because with a diagnosis of CC in people with T2D, cytotoxic activity of NK cells is almost four times lower compared to those with diabetes without cancer.

To summarize, we can conclude that impaired NK cell activity is probably associated with increased susceptibility to CC in subjects with T2D. Normalization relating functioning of these cells may become one of the goals in both antidiabetic and anticancer therapy. The beneficial effect of NK cells immunotherapy added to chemotherapy of CC has already been described (Iovino et al. 2011).

In conclusion, the study of NK cells and impaired function of these cells is entirely original, and its purpose is to explain the increased incidence of CC in patients with T2D. The available international literature provides no data on this topic. Further discoveries in the field of innate immunity will probably contribute to a better understanding related with the pathophysiology of CC in T2D and may lead to introducing new experimental forms of preventive medications (regardless of the number of currently proposed treatments) (Jaime-Ramirez et al. 2011; Sakakibara et al. 2011; Zheng et al. 2011) in people facing the greatest risk associated with the occurrence of this form of cancer in this ever-increasing risk group of patients.

Conclusions

1. It has been shown that a trace activity of NK cells in subjects with T2D, as well as CC, suggests the legitimacy of the need to determine this activity in T2D;
2. The discovery relating the low level of cytotoxic activity of NK cells in subjects with T2D may suggest an increased risk of CC in this group of patients;
3. Frequent screening tests towards CC in subjects with T2D, especially in subjects with coexisting insulin resistance, seem to be of a great purpose.

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