PROBLEMS OF CANCER TREATMENT

PART I
THEORY OF TREATMENT BASED ON KNOWN MECHANISMS OF ANTICANCER IMMUNOLOGICAL RESPONSES

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Summary: Various processes, taking place both in cells and in their environment, are linked to carcinogenesis. This paper aims at recalling the complex mechanisms of oncogenesis, with particular attention paid to responses of the immune system. In development of solid tumours, leukaemias and lymphomas several common stages can be noted. A neoplastic disease cannot be understood considering only phenomena of genetic mutations. Neoplastic cells are characterised by an extensive antigenic variability and resistance to apoptosis. The cells create around them a microenvironment which protects them from defensive activity of the host. In the paper we present the recognised mechanisms of anti-neoplastic defense as well as several elements allowing the solid tumours and leukaemias to escape from the immune surveillance. The generally accepted treatment of tumours aims at reducing numbers of tumour cells. Following resection of a tumour, radiotherapy or chemotherapy, the parallel or consecutive stage of treatment was found to involve an increase in number of clones of immune system cells. One of the ways in which the immune system can be activated involves autovaccination of the host with own neoplastic cells in an apoptosis. However, attempts of such a therapy frequently brought no expected results due to blocked activity of cytotoxic cells. Therefore, the subsequent stage in activation of the immune system should involve elimination of the tumor-mobilized blockade of the system. Attempts toward this aim include neutralization of the tumour-blocked cytotoxic properties of defensive cells, first of all T lymphocytes. The recognized mechanisms of blocking T cells activity in the PD-1/PD-L1 system or due to inhibition of activation by CTLA-4 molecule provided rationale for development of effective tumour immunotherapy approaches.

Keywords: targeted therapy, lung cancer, leukaemias, tumour microenvironments, suppressor cells, tumour markers

Abbreviations: **ADCC** – *antibody dependend cell cytotoxicity*; **AML** – *acute myeloid leukaemia*; **APC** – *antigen presenting cell*; **BAL** – *bronchoalveolar lavage*; **BTLA** – *B,T lymphocyte attenuator*, CD272; **CLL** – *chronic lymphocytic leukaemia*; **CML** – *chronic myeloid leukaemia*; **CRAM** – *chemokine receptor on activated macrophages* (known also as HCR or CCRL2); **CTLA-*4*** – *cytotoxic T lymphocyte antigen*, CD152; **CTLs** – *cytotoxic T lymphocytes*; **DAMPs** – *danger (damage) -associated molecular patterns*; **DCs** – *dendritic cells*; **EGFR** – *epidermal growth factor receptor*; **EMT** – *epithelial mesenchymal transition*; **EOC** – *epithelial ovarian cancer*; **EpCAM** – *Epithelial cell adhesion molecule*; **Foxp3** – *forkhead box P3-transcription* – transcription factor Foxp3; **HMGB1** – *high- mobility group box-1*; **HSP** – *heat shock protein*; **IL** – interleukin; **LAG-3** – *lymphocyte activation gen-3*; **M1,M2** – macrophage subpopulations M1, M2; **MAGE** – *A3 melanoma associated antigen*, **MDM2** – *murine double minute2* – mouse protein MDM2 (E3 ubiquitin ligase); **MDSCs** – *myeloid derived suppressor cells*; **MHC** – *major histocompatibility complex*; **MMP** – *metalloproteinase*; **MUC1** – *Mucin 1, cell surface associated*; **NK** – *natural killer cells*; **NKT** – *natural killer T cells*; **NO** – *nitric oxide*; **PD-1** – *programmed cell death protein1*, CD279; **PD-L1, PD-L2** – ligand for PD-1; **COPD** – *chronic obturative pulmonary disease*; **ROS** – *radical oxygen species*; **TAM** – *tumour associated macrophages*; **TAN** – *tumour associated neutrophils*; **TATE** – *tumour associated tissue eosinophils*; **TCR** – *T cell receptor*; **TGFβ** – *transforming growth factor β*; **Th** – *T helper lymphocytes*; **Tc** – *T cytotoxic lymphocytes*; **TIL** – *tumour infiltrating lymphocytes*; **Tim3** – *mucin domain-containing molecule-3* – transmembrane immunoglobulin and mucin 3; **TIMP1** – *tissue inhibitors of metalloproteinase-1*; **TNF** – *tumour necrosis factor*; **Tregs** – *regulatory T lymphocytes*

INTRODUCTION

Aside from cardiovascular diseases, tumours represent the most frequent cause of death. This study aims at summing up the till now available chances for treatment and presenting principles of immunotherapy, which may asssist the classical therapy of tumours. At present a tendency is observed of diagnosing not only the tumour type and its markers but also condition of the host immune system which may point to an individual prognosis of the disease course [59]. This paper aims not at providing details of the treatment but at reviewing problems associated with therapies planned to control and to activate immune system of patients.

HOW TUMORS ARISE – THE EXAMPLE OF LUNG CANCER AND LEUKAEMIAS

Neoplastic tumours develop in several stages, well described in, e.g. colorectal carcinoma in humans and dermal melanoma in mice [3, 16, 78].

Post-translational modifications of histons, methylation and demethylation of bases in nucleic acids, similarly to changes in nucleotide sequence, translocation of chromosomal segments lead to activation or inactivation of numerous genes. The epigenetic changes may stably re-program cells, which aquire phenotype of neoplastic cells [111]. Cells with mutations, transformed ones, may compete

with each other, leading to cell selection. Gradually forming a small focus or intraepithelial lesions, e.g. a dysplatic focus. Developing tumors consist not only of transformed cells but also of numeorus untransformed cells of their sublayer and immune system. At the early stages of oncogenesis host's immune system recognizes and responds to presence of the altered cells while further development of the tumour depends on alterations in immunogenic properties of cancer cells [91]. Survival of the transformed cells is linked to their adaptive response to environmental conditions [106].

Most of tumours contains a variable fraction of cells manifesting markers of stem cells [113], recently with increasing frequency termed the tumour propagating cells. The cells manifest genome instability. High levels of oxygen reactive forms in the cells result in injuries of DNA, leading to accumulation of consecutive chromosomal aberrations [99]. Origin of the cells remains unclear although they are identified using surface markers, including, among other, CD133, CD44, CD29. Considering high variability of neoplastic cells it remains disputable if such cells can be distinguished [136].

AVOIDANCE OF TUMOUR RECOGNITION BY CELLS OF IMMUNE SYSTEM – THE EXAMPLE OF LUNG CANCER

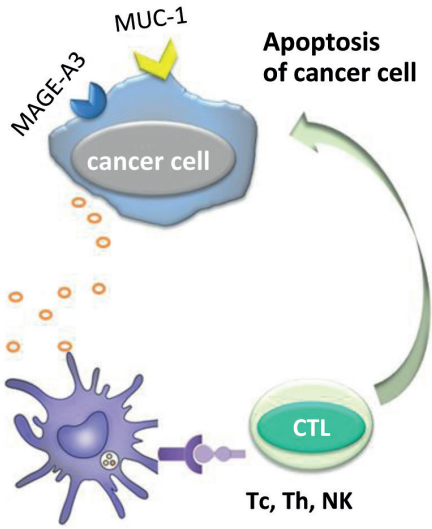
Lung cancer presents a typical example of a solid tumor, on which the above mentioned disturbances can be characterised. Also the epidemiological data justify a broader presentation of the tumour The tumour provides the principal cause of death due to malignant processes in both genders [31]. The respective annual incidence exceeds worldwide 1 600 000 while the combat with the disease is won by just around 15% patients. The very rapid and extremely deceitful course of lung cancer causes that at the moment of diagnosis over 70% of the tumours manifests a significant advancement, making impossible causal, particularly operative treatment [20, 31]. Surgery remains the only effective method of treatment in most of solid tumours, including non-small cell pulmonaty cancer, apart of few histological types which are sensitive to chemotherapy, like, e.g., small-cell lung cancer [52, 57]. The lack of possibilities and inefficacy of classical therapeutic approaches justifies an extensive search for new therapeutic approaches including a targeted therapy and an immunomodulatory treatment.

Similarly to other tumours, in lung cancer the primary antineoplastic response is effective at the phase of a very early oncogenesis [1, 9]. The hypothesis of immune surveillance of Burnet and Thomas (1957) assumes a destruction of altered, pathological cells and a continuous protection of the host against neoplasia. Currently, tumour development is thought to be significantly modified by the immune system. In the process, an important role is ascribed to tumour-

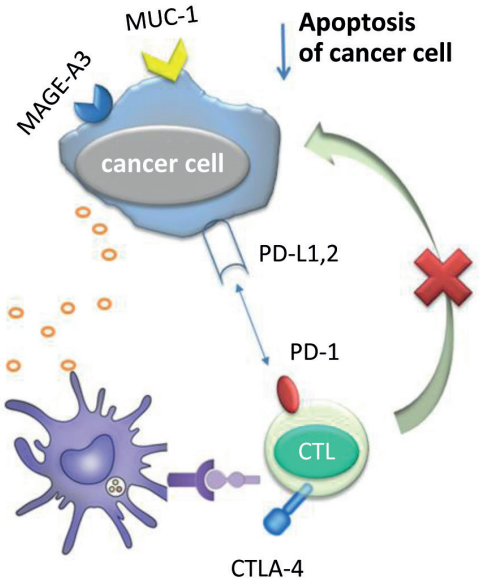
infiltrating inflammatory cells [30]. However, the primarily effective immune surveillance becomes altered in a very refined way by a weakened recognition of cancerous cells, a reduced function of antigen presentation and by a prevalence of an inhibitory over the activating immune response [1, 26]. The principal mechanisms by which a tumour sneaks through the immune surveillance are presented in fig. 1.

Most of solid tumours, including in particular lung cancer, affects patients of an advanced age, in whom a cummulation developes also of harmful effects of external conditions, including tobacco smoke, the principal risk factor for development of lung cancer [22, 49]. The investigated topics include also phenomena linked to effects of oxydative stress, which accelerate physiological processes of senescence, including senescence of cells in immune system, termed „inflamm-aging" [33]. Accvording to suggestions of Franceschi, in the course of senescence the decaying cells release stress factors, which additionally stimulate the immune system. This results in a certain exhaustion of defensive functions. Senescence of the immune system is associated with increased concentrations of IL-8, IL-6, TNFα, and, in parallel, decreased concentrations of IL-2 are detected [33]. T lymphocyte polarizations is shifted toward Th2 type responses. Phenotype of NK cells undergoes a change, accompanied by a reduction in their cytotoxic potential. The senescence of immune system is expressed in a loss of its ability to recognise foreign and own antigens. The sphere of such disturbances closely resembles alterations observed in the course of neoplastic diseases [33, 36].

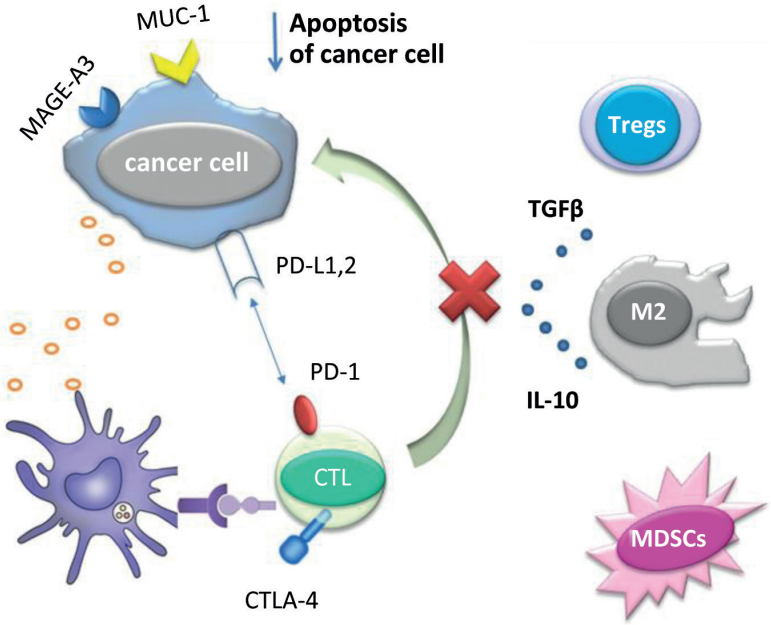
FIGURE 1. Scheme of mechanism of a cytotoxic anti-neoplastic reaction and its inhibition
a) Cytotoxic cells (CTLs) following stimulation induce apoptosis of a neoplastic cell



b) Inhibition of lymphocytes by PD-1 and CTLA-4 molecules prevents against a cytotoxic reaction



c) In addition, the cytotoxic response becomes controlled and an immune tolerance develops with involvement of cells with controlling properties: Treg, M2 and MDSCs and cytokines, TGFβ, Il-10



MARKERS OF NEOPLASTIC CELLS ON THE EXAMPLE OF LUNG CANCER

Cells of lung cancer are characterized by poorly defined and variable antigenicity, which causes extensive histopathological heterogenicity. The best known antigens, the presence of which is reproducibly detected include MAGE-A3 (melanoma associated antygen) and MUC1. MAGE-A3 is detected in 35-50% patients with non-small cellular lung cancer, mainly of the squamocellular type. Expression of the antigen is linked to a poor prognosis. MAGE-A3 recognised by cytotoxic cells provides ground for production of numerous vaccines [88]. In clinical trials the need is stressed for confirmation of expression of the antigen on tumour cells before treatment. Glycoprotein MUC1 is commonly present on cancer cells in glandular type tumours and its expression also is linked to an unfavourable prognosis. Ita extracellular domain, MUC1 is strongly immunogenic, proangiogenic, it intensifies migration of tumour cells and their resistance to apoptosis [155]. The subsequent therapeutic target involves receptor for epithelial growth factor (EGFR) commonly present on cells of lung cancer [77]. Its activity can be inhibited using CIMAvax vaccine, taking advantage of the antibody specific for EGFR [89]. Until now, few recognised epitopes of cancer cells were described [121], such as, for example, peptides in patients with melanoma [21, 46] and in ovarian cancer [114]. Due to mutations taking place in cancer cells development of a solid tumour is accompanied relatively dynamic appearance of new antigens, changes in surface markers develop or the antigens become masked. The cancer cells become "unrecognisable" for the immune system and, therefore, no conditions exist for development of the acquired immunity mechanisms. Nevertheless, as recently demonstrated, the therapies applied in lung cancer, chemotherapy, radiotherapy or even a targeted therapy, promote stimulation of a reaction due to „uncovering" the antigens and formation of neo-antigens in effect of a certain cellular stress [145]. The phenomenon carries significant therapeutic implications for the currently executed clinical trials in which immunotherapy is accompanied by chemotherapy [138].

DEVELOPMENT OF SYSTEMIC TUMOURS ON THE EXAMPLE OF B-LYMPHOCYTE LEUKAEMIA

Also systemic tumours of leukaemia/lymphoma type escape from immune control. Their very early stages remain unknown but considering behaviour of cells in one of leukaemias (chronic B-cell laukaemia) [90, 146], certain hypotheses can be suggested. Normal B lymphocytes of $CD19^{+}CD5^{+}$ subpopulation continuously

produce antibodies and in that function require no activation by T lymphocytes. Cells of the subpopulation provide origin for the leukaemia. Most of circulating leukaemic lymphocytes are stopped in the cell cycle at the G0/G1 phase and, therefore, accumulation of increasing numbers of lekaemic cells is thought to result from their resistance to apoptosis induced in them during normal senescence rather than from their frequent divisions [17, 19]. Elongation of the cell cycle leads to cummulation of mutations in the cells and to manifestation of traits later characterizing leukaemic cells [69]. The mutated B $CD19^{+}CD5^{+}$ lymphocytes compete with each other and used to select individual clones of the cells, already representing the leukaemic hyperplasia.

In normal development, the microenvironment of bone marrow contains interactions of B lymphocytes with the produced by stromal cells ligands, which maintain in the bone marrow niche the progenitor cells with expression of CXCR4 receptor and c-kit [32, 95]. Similarly to progenitor cells, leukaemic cells manifest expression of CXCR4 receptor (CD184] [10, 85]. Cells of bone marrow stroma and nurse-like cells of bone marrow continuously secrete the CXCL12 (SDF-1] ligand and in this way attract and maintain in the bone marrow niche leukaemic B lymphocytes [140] and inhibit their apoptosis. The persistent harbouring of $CD19^{+}CD5^{+}$ lymphocytes in bone marrow causes that after some time the leukaemic cells no longer are able to produce antibodies but continue to survive in the environment. Moreover, stromal cells protect leukaemic lymphocytes from apoptosis induced by, e.g., chemotherapy. A similar environment in lymph nodes is provided to B-CLL cells by CRAM receptors, present of their surface [14, 75].

ALTERATIONS IN TUMOR MICROENVIRONMENT

The accumulated in a specific place cells with a modified phenotype begin to produce proteases, which hydrolyse the surrounding extracellular matrix (matrix metalloproteinases, MMP) and tissue inhibitors of metalloproteinase 1 (TIMP-1] [48, 80, 127], which leads to progression of lesions in the pre-invasive cancer (*carcinoma in situ)*. The proteases degrade components of the intercellular matrix and basement membranes of epithelium, which promotes invasion of neoplastic cells and their appearance in the circulation [39, 123]. MMP represent endoproteases synthetised in precursor forms and activated by proteases such as plasmin. They include, i.a., collagenases (MMP1], gelatinases (MMP2, MMP9] and membrane metalloproteases (MMP14). Overexpression of the proteases is frequently linked to poor prognosis for the patient [68, 127].

Tumour cells induce ingrowths of blood vessels into their region. Blood supply of neoplastic tumour condition inflow of glucose to cancer cells usually

manifesting glycolytic metabolism [41], which represents an important stage in further development on the lesion. This results in a more rapid growth of the tumour and in presence of accompanying cells of chronic inflammatory process, macrophages and lymphocytes. The tumour-infiltrating lymphocytes T (tumor infiltrating lymphocytes, TILs) can be expected to recognise neoplastic antigens since on their surface activation markers appear, e.g. cytotoxic lymphocytes T $CD8^+$ manifest surface expression of HLA-DR and CD38, which represents an important stage in further development of the lesion [71]. Nevertheless, the cells do not function effectively as tumour-combatting cells.

A decay of neoplastic cells can also be observed. The decaying cells are rapidly recognised and phagocytosed, including also myeloid dendritic cells (mDC). Dendritic cells manifest their capacity to cross-present antigens [64, 92, 101, 108, 135], or to present epitopes of phagocytosed cells, in this case epitopes of cancer cells with MHC class I antigens [55, 102]. Cells distinct than mDC do not manifest such properties. This is important inasmuch as neoplastic cells manifest an altered mechanism of presenting their own antigens and in such a situation the latter remain unrecognised. Thus, cross-presentation involves the manner due to which such behaviour on neoplastic cells does not allow them to bypass the immune recognition and epitopes of neoplastic cells can in this way be presented to virgin lymphocytes T. Following phagocytosis of decaying neoplastic cells, dendritic cells migrate to lymph nodes [104] and there, together with MHC class I, present the neoplastic epitopes to virgin lymphocytes T which flow through the lymph node [54]. However, such presentation of antigens by mDC present within a neoplastic tumour can be blocked [87, 142]. On the basis of our own studies we found that alterations in the systemic circulation differ significantly from those in the vicinity of a tumour [25]. Thus, antigens of neoplastic cells are well recognised by mechanisms of inborn and acquired (specific) immunities out of the site of the neoplastic tumour but neoplastic cells significantly modify control of immune processes in their surrounding [74] and in this way protect neoplastic cells from attack of immune system cells.

PROCESS OF APOPTOSIS

Neoplastic cells are relatively resistant to extracellular signals of apoptosis [40, 86, 120]. Apoptosis develops along two principal pathways [35]: (a) pathway of inner origin with involvement of a recognised danger, with participation of p53 protein, leading to activation of apoptotic process with involvement of mitochondria, inhibited expression of genes the products of which exert antiapoptotic effects, e.g. Bcl2, survivin [98] and (b) pathway of external origin, with activation of

proapoptotic surface receptors (death receptors, DR) [2, 65, 151]. This second pathway can be activated from outside of the cell by proapoptotic ligands of TNF protein superfamily, like, e.g. Fas ligand (FasL) and TRAIL [2, 93]. The ligands bind to proapoptotic receptors on the surface of tarnet (cancer) cells and transmit signal for activation of procaspase-8, and this activates subsequent caspases responsible for the course of apoptosis. Occasionally, this extraneous activation of apoptosis amplifies in the cell the inner signalling of apoptosis with mediation of Bid proteins of Bcl family. Caspase 8, activated along the pathway of external origin splits the Bid protein, which in this form becomes transplaced to mitochondria, initiating the internal pathway of apoptosis [155]. The two pathways converge at the stage of effector caspases, which proteolytically hydrolyse and/or activate important cell proteins (e.g. DNase which induces fragmentation of DNA).

TRADITIONAL APPROACH TO TUMOUR TREATMENT: CHEMOTHERAPY, RADIOTHERAPY AND THEIR ROLE IN IMMUNOTHERAPY

Routinely, cancer tumors are treated by surgical removal, chemotherapy and radiotherapy. Additionally, in some patients immunotherapy is applied, involving administration of monoclonal antibodies directed at antigens of cancer cells. In presence of complement and cells with receptors for the Fc fragment of immunoglobulins they may directly activate death of target cells. This is an important stage of therapy, leading to reduction in cancer cell number and after such procedures many patients manifest transient improvement. However, one should be prepared for subsequent proliferation of the remaining in the patient cancer cells which propagate the tumour or for development of a new tumour, activated by chemotherapeutic agents or by radiotherapy. Chemotherapeutic agents and radiotherapy lead to DNA injuries but neoplastic cells contain more efficient than “healthy” cells mechanisms of DNA repair, which in consequence may lead to further mutations and tumour development [83], as well as to multidrug resistance [50, 82, 125]. In the course of tumour development more than 50% tumour cells due to point mutations loose function of p53 protein [149, 150], or of MDM2 protein. The MDM2 (Murine Double Minute 2) protein, a repressor of p53, undergoes a deregulation [144, 147], with the resulting appearance of various tumour types. They do not respond to therapies inducing the inner pathway of apoptosis, evoked by chemotherapeutic agents or radiotherapy. On the other hand, the external pathway of apoptosis functions independently of activity of p53 protein. Therefore, it can be employed for induction of the programmed death in neoplastic cells by T lymphocytes.

Surgical removal of a tumour frequently is assisted by chemotherapy and radiotherapy. Radiotherapy is targeted at the tumour site or lymph nodes which may be occupied by tumour cells. Such procedures can be precisely targeted at neoplastic foci and they do not significantly damage the patient's immune system. Local irradiation is followed by additonal systemic interactions involving release of neoplastic antigens and the so called damage-associated molecular patterns (DAMPs). The molecules released from the damaged cells include, i.a., DNA, ATP, heat-shock proteins [HSP), high-mobility group box 1 HMGB1 proteins, components of mitochondria and extracellular heparin sulphates [73]. Radiotherapy may also increase absorption and cross-presentation of neoplastic antigens [37].

Another manner of antineoplastic therapy involves chemotherapy used in solid tumours before and after surgery (termed the neoadjuvant and adjuvant therapy) and in the first choice in lymphomas and leukaemias. Several effective combinations of chemotherapeutic agents were selected [11, 61], occasionally leading to a complete cure.

Chemotherapy used to be applied repeatedly. The principal problem involves low specificity of most frequently applied chemotherapeutic agents. Nevertheless, in some cases chemotherapeutic agents augment antineoplastic resistance [62, 124, 109). A great energy is devoted to selection of the more specific drugs on the basis of knowledge involving genetic lesions, most frequently taking place in specific types of tumours [105, 122, 152]. For example, in CML in which neoplastic cells manifest a translocation leading to development of a fusion BCR-ABL gene, a specific inhibitor of tyrosine kinase termed imatinib (commercial name of Gleevec) provides good clinical effects in chronic phase of the disease [7, 97, 156]. However, such direction of the therapy manifests limitations reflecting extensive variability of genomes in the neoplastic cells [69] and in the patients neoplastic cells may appear which are resistant to the specific drug [156]. In such situations new generations of drugs are used and new sequences of their administration, which occasionally yields positive results. Thus, the system involves permanent search for new drugs, acting on the continuously modifying in the patient neoplastic cells.

In the search for specificity of the applied drugs monoclonal antibodies are used, which decrease mass of the tumour. Occasionally, these are chimeric (human/mouse) or humanised antibodies (human antibodies with mouse fragments in the hypervariable regions, binding the antigen), directed at surface antigens (markers) on neoplastic cells. The aim is similar: to decrease number of neoplastic cells using specifically targeted antibodies, with possibly minimum damage to the immune system. The classical antibody used in antineoplastic therapy involves immunoglogulins directed at CD20 protein, specifically expressed in the surface of B lymphocytes, inact or neoplastically transformed (leukaemias and B-cell lymphomas). The clinically used monoclonal anti-CD20 antibodies include

rytuxymab, human-mouse chimeric antibody (RTX) [110, 141], ofatumumab, human monoclonal antibody directed against both the small and the large extracellular loop of CD20 antigen [5], glycosylated anti-CD20 antibodies [6, 100, 128].

In presence of complement proteins in the plasma and in intercellular fluids binding of the antibody to a neoplastic or a normal cell induces in such a cell apoptosis along the exogenous pathway. Also cells of immune system recognise cells which bound antibodies due to presence of immunoglobulin heavy chains on their surface. The „protective" cells, such as NK cells, neutrophils, monocytes carry surface receptors for Fc parts of immunoglobulin chains, bind to the „labelled" cells and kill them, which is defined as antibody-dependent cell cytotoxicity (ADCC). Examples of antibodies used in tumour immunotherapy are presented in table 1.

TABLE 1. Examples of antibodies clinically used for destruction of neoplastic cells

ANTIBODY	ANTIGEN	TARGETED TUMOUR	TYPE OF ANTIBODY
Rutiximab	CD20	B-CLL*, NHL	chimeric
Alemtuzumab	CD52	B-CLL	humanised
Ibritumomab tiuxetan	CD20	NHL	mouse with ^{90}Y or ^{111}In
Tositumomab	CD20	NHL	humanised
Cetuximab	EGFR	colorectal carcinoma, head/neck carcinoma	chimeric
Panitumumab	EGFR	colorectal carcinoma	humanised
Catumaxomab	CD3, EpCAM	exudate of EpCAM to peritoneum with neoplastic cells	mouse/rat hybrid
Ofatumumab	CD20	B-CLL	humanised
Pertuzumab	HER2	breast carcinoma	humanised
Obinutuzumab	CD20	B-CLL, B-cell lymphomas**	humanised, glycosylated Fc

* – exerts cytotoxicity mainly due to activation of complement, ** – exerts cytotoxicity mainly due to ADCC reaction

As can be found, currently a physician in the clinic has at his disposal several antibodies against various tumour cells, e.g. anti-CD52 (Alemtuzumab, leukaemias, lymphomas) [112], anti-HER2 (Herceptin, breast carcinoma), antibody specific for the receptor for human epidermal growth factor (EGFR) type 2 (Erbb2) of for the receptor for epidermal grpwth factor, EGFR1 (Cetuximab, Panitumumab, colorectal

carcinoma, lung carcinoma) [56]. Also for a long time therapies have been applied in which antibodies administered to patients were linked to isotopes (Ibritumomab). Attempts were also made to use bacterial toxins or to amplify with them effects of antibodies [42, 133].

As already mentioned above, the early stages of therapy aim at decreasing mass ot the tumour, which in addition partially unblocks systemic inhibition of immune defense, e.g. unblocks maturation of T lymphocytes in the thymus [63, 66, 67]. This probably results from competition of leukaemic cells with normal lymphocytes for space in the thymus (12, 12a). The thymus provides the site for maturation of T lymphocytes, including Treg, also in an adult body [12, 96].

DISTURBANCES IN IMMUNE SYSTEM IN SOLID TUMOURS AS WELL AS IN LEUKAEMIAS AND LYMPHOMAS

Following a routine anti-neoplastic treatment, remains of cancer cells still reside in the host. They should be removed by immune system of the host, mainly by lymphocytes T. However, the remaining neoplastic cells block the directed against them local action of cytotoxic lymphocytes.

Lymphocytes T continuously monitor „correctness" of cellular protein structures, which allows them to remove pathogens and maintain body homeostasis. In order to be able to recognise the presented epitope (antigenc determinant), created by a protein fragment of 8-10 amino acids together with MHC class I the lymphocytes T must become activated to the effector response. For this purpose they require two signals. The first involves recognition of the epitope and binding by receptor on T lymphocyte (TCR) of antigenic determinant, presented by antigen presenting cells (APC) together with histocompatibility antigens. The other signal is provided by parallel interaction between CD28 protein on a lymphocyte T with ligands B7 (B7.1, CD80 and B7.2, CD86] in APC cell [126]. The process is termed a co-stimulation [15]. The interactions result in activation of the recognising the epitope lymphocytes T, $CD4^+$ and $CD8^+$, which includes their proliferation and ability to induce apoptosis in the target cells or activation of dendritic cells [DC) using cytokines.

The process inverse to co-stimulation involves inhibition of co-stimulation or co-inhibition, taking place with involvement of at least two groups of molecules: (a)superfamily of immunoglobulin receptors [immunoglobulin super family, IgSF) and (b) superfamily of TNF receptors (TNFR super family) [15]. The first group of molecules (IgSF) resembles in structure chains of antibodies with duistinguishable IgV and IgFc domains [153]. The group encompasses stimulatory co-receptors CD28, ICOS, and co-inhibitory receptors: CTLA4, PD-1, LAG3, TIM3, BTLA, VISTA, CD160. Co-receptors of the second group (TNFR SF) exhibit stimulatory properties and the group includes molecules of GITR, OX-40, CD30, CD40 and

4-1BB [116]. The mentioned co-receptors fulfil also important functions in viral infections (CD40], e.g. in influenza infection [157]. At present, antibodies are produced directed against co-stimulatory molecules (dacetuzumab, anti-CD40) and co-inhibitory proteins, aiming at influencing their control over immune responses [45]. The antibodies are tested using both in vitro and animal models. Clinical trials have also been started on patients. Such a local influence on activity of the immune system used to be termed a control of checkpoints of lymphocyte T activation.

As mentioned above, co-stimulation of lymphocyte T incompasses recognition of the epitope and it is required for induction of a series of cell divisions of the recognising lymphocyte, its capacity to induce apoptosis in the target cell and for expression of cytokines. Of the recognition signal requires more time, e.g. due to presence of cancer cells in vicinity of T lymphocytes, on the surface of activated lymphocytes T CTLA-4 protein (cytotoxic T lymphocyte antygen 4, CD152) appears. CTLA-4 interacts with molecules B7 in dendritic cells and the remaining APC, manifesting a higher affinity for them than CD28 protein does. Thus, CTLA-4 protein preferentially binds to the B7 molecules, blocking binding of CD28. Resting lymphocytes T manifest low expression of CTLA-4 protein or are devoid of it while higher expression of the protein on lymphocytes T develops following their activation [38].However, interaction with CTLA-4 does not transmit the co-stimulatory signal, as it happens in effect of the interaction with CD28 and in such a situation lymphocyte T does not acquire cytotoxic properties toward the target, cancer cells [72, 119]. The appearance of CTLA-4 on activated lymphocytes T involves a regulatory process, which inhibits the extending activation of lymphocytes T and which depends on several variables [118]. In normal conditions such appearance prevents processes of allergy, hypersensitivity due to the extended in time process of immune „defense". However, in parallel, this is also a process which inhibits defense against tumours, due to numerous, similar to CTLA-4 co-inhibitory molecules which appear on lymphocytes T $CD4^+$, on around every other lymphocyte T $CD8^+$, on Treg lymphocytes, but also on lymphocytes B and NK lymphocytes [4, 15, 119, 134]. The CTLA-4 molecules are mainly accumulated in cytoplasm of the activated cells. Appearance of the molecules on the lymphocyte surface is controlled by interactions with microenvironment which surrounds the cells [148]. Another system inhibiting activity of lymphocytes was also described, the PD-1/PD-L1 system. Lymphocytes T activated in a neoplastic process or a non-neoplastic process release INFγ, which induces production of ligands: PD-L1 (CD274] and PD-L2 [CD273] by cancer cells, if they exist in the neighbourhood [129]. Coupling of PD-L1 or PD-L2 ligands to lymphocytes T with expression of co-inhibitory receptors, defined as PD-1 (Programmed cell Death protein 1) [13], results in inhibition of the stimulation, leads to blocked production of cytokines, less extensive proliferation of lymphocytes and less pronounced lysis of target cells. The mechanism results in local anergy of lymphocytes T in tumours.

The cytotoxic cells forming the CTLs (cytotoxic T lymphocytes) pool encompass Tc lymphocytes ($CD8^+$), NK cells, NKT and also some Th lymphocytes [$CD4^+$). In our own studies the altered numbers of cytotoxic lymphocytes were confirmed within lungs in patients with lung carcinoma, as compared to healthy individuals. A profile of cells present in bronchoalveolar lavage (BAL) fluid was prepared and lymphocyte subpopulations were examined by flow cytometry. In patients a significantly higher proportion of lymphocytes T and of $CD8^+$ lymphocytes and a reduced percentage of $CD4^+$ lymphocytes and a reduced ratio of lymphocytes T $CD4^+/CD8^+$ [25, 47]. In the same patients lymphocyte populations were examined in peripheral blood and reverse alterations were detected: proportion of $CD8^+$ lymphocytes in the blood was lower in patients with lung carcinoma than in normal individuals while the Th/Tc ratio was higher [25, 47]. Similar results were obtained in a study of very numerous group of 140 patients, in whom lymphocyte typing was performed using immunocytochemistry [23]. The assumption seems justified that lymphocytes defined in BAL reflect composition of TIL infiltrate in the tumour. Importantly, our observations were made before start of treatment and they illustrate condition which probably becomes altered during development of the cancer and, even more, during therapy. Numerous studies conducted in patients with solid tumours, including lung carcinoma confirm positive prognostic significance of $CD8^+$ lymphocytes infiltrate at the site of a tumour [28]. The anti-neoplastic reaction was demonstrated also to be modified through the effect on migratory potential of lymphocytes in the extracellular matrix of a tumour. The study of H. Salmon et al. presence of some mechanical barrier around a focus of cancerous cells which restricted access of inflammatory cells and document functional role of extracellular matrix in the process [115].

Similarly to other neoplastic hyperplasias, in cancer carcinoma presentation of antigens by APC cells manifests a key importance. The mechanism of lymphocyte activation is similar and similar is the role of the suppressory CTLA-4 molecule. CTLA-4 appears on effector T lymphocytes in the environment of the cancer and it blocks transmission of the signal from the APC cell, in this way blocking the cytotoxic effect. On the other hand the function of CTLA-4 on T regulatory cells (Treg) in tumour environment is distinct [8, 29, 76, 117]. CTLA-4 is continuously present on Treg and augments suppressory function of the cells. The role of regulatory cells has been well described and in several studies the cells were proved to be present in high numbers in neoplastic infiltrates and to play a significant role inhibiting anti-neoplastic response [27]. The suppressory function of Treg in tumour environment is linked to activity of Foxp3 transcription factor, also used for identification of Treg. In numerous studies on pulmonary carcinoma a negative prognostic significance of presence of Treg with expression of Foxp3 was documented [60, 103, 137]. Confirmation of Foxp3 expression in regional lymph nodes, even of clinically free of metastases provides a negative prognostic factor [43].

ROLE OF NEOPLASTIC CELLS IN INHIBITION OF IMMUNE SYSTEM ACTIVITY

Anti-neoplastic response becomes actively altered by cancer cells [34, 70, 71]. The alteration takes place through the mechanism of modification affecting the environment in which the cancer develops, through secretion of suppressory cytokines, which stimulate cells which inhibit inflammatory response and through an increased expression of ligands for receptors of suppression and apoptosis (PD-L1, PD-L2, Fas-L) on cytotoxic cells [53, 58, 139]. In inhibition of immune system response to tumour development disappearance of cytotoxic lymphocytes and inhibition of their function are significant. Among other, an increased prevalence of apoptosis takes place among protective cells. One of the mechanisms involves the Fas/FasL receptor pathway. In patients with cancer the circulating $CD4^+$ and $CD8^+$ lymphocytes exhibit markedly augmented expression of Fas receptor, which points to their susceptibility for apoptosis, which can be noted after binding the ligand on cancer cells [24, 51]. In our studies we documented an increased number of lymphocytes with expression of Fas in tobacco smokers and patients suffering from pulmonary chronic obturative disease (PCOD), which may point to suppression of the immune system and an augmented risk of pulmonary cancer development [24, 49].

The anti-neoplastic defense engages also cells of myeloid line but hey exhibit mainly suppressive functions. In recent years two populations of macrophages, M1 and M2 were distinguished. M1 macrophages fulfil mainly immunostimulatory functions producing as effector cells significant amounts of pro-inflammatory cytokines (among other they produce IL-12] and participate in phagocytosis. In turn, M2 macrophages exhibit immunosuppressory functions, they promote angiogenesis and modelling of their environment [84]. In the environment of a solid tumour M1 macrophages stimulated by LPS and IFN-γ activate the anti-neoplastic response but, unfortunately, they are predominated by M2 cells which form the main population among tumour associated macrophages (TAM) and co-operate with cytokines IL-4, IL-10, IL-13 and TGFβ [9, 81, 94]. Moreover, the M2 population is known to co-operate with regulatory cells and IL-17 interleukin, capable of controlling immune responses [79]. The TAM population can be examined in dissected lung cancers while in advanced tumours macrophages can be obtained using the BAL approach. Employing this technique a significantly „handicapped macrophage function was demonstrated in pulmonary carcinoma in response to interferon" [18].

A pronounced suppressor function in tumour microenvironment is played by myeloid-derived suppressor cells (MDSCs), activated by numerous locally secreted cytokines. The MDSCs are capable of controlling immune responses, they modulate lymphocyte function by production of nitrogen oxide (NO), free

oxygen radicals (ROS), TGFβ and PGE2 and they reduce availability of amino acids for effector cells by competitive usage of substrates [131, 132]. Function of MDSCs is linked also to the epithelial-mesenchymal transformation (EMT) which promotes penetration of cancer cells and angiogenesis [130]. Even if significance of macrophages and MDSCs for immune responses has been defined no specific treatment is available which would restrict their function while attempts to inhibit them by enzymatic approaches are at the stage of studies.

Cancer and inflammatory cells in the tumour environment are capable of releasing substances of immunosuppressive action. One of the best known cytokines exerting such an influence involves the transforming growth factor, TGFβ, identified in augmented concentrations in tumours, in cultures of tumour cells and in BAL fluids originating from patients with lung cancer [9]. The main suppressive activity [44] affects function of NK cells and cytotoxic T lymphocytes, differentiation of helper cells toward Th2 and maintenance of Treg differentiation [143]. In addition, cancer cells due to loss of receptors for the factor become insensitive to its inhibitory function. The function of TGFβ is labile, dependent on local conditions and in first stages of cancer development to favourable for the host [107]. Currently attempts continue to take advantage of new forms of immune modification in therapy, closer described in the second part of this paper.

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