Review

Prediction of Response in Cancer Immunotherapy

DAINIUS CHARACIEJUS[1,2], JOHN J.L. JACOBS[3], VITA PAŠUKONIENĖ[4], NIJOLĖ KAZLAUSKAITĖ[4], VITA DANILEVIČIŪTĖ[1], MYKOLAS MAURICAS[2] and WILLEM DEN OTTER[3]

[1]*Faculty of Medicine, Vilnius University, M.K.Čiurlionio 21, Vilnius, Lithuania;*
[2]*Centre for Innovative Medicine, Žygimantų 9, Vilnius, Lithuania;*
[3]*VU University Medical Centre, de Boelelaan 1117, Amsterdam, The Netherlands;*
[4]*Institute of Oncology, Vilnius University, Santariškių 1, Vilnius, Lithuania*

Abstract. *This review focuses on the relationship between pre-treatment immune parameter values and outcome of immunotherapy of cancer patients. The evidence presented in this review suggests that there is a relationship between pre-treatment immune parameter values and survival of cancer patients treated with immunotherapy. Tumour-infiltrating immune cells may have a predictive value for immunotherapy, but predictive power might be obtained from peripheral blood leukocytes. Use of peripheral blood may be preferable due to the convenience of collection and analysis compared to using tumour-infiltrating cells. In vivo numbers of cells of the immune system correlate better with clinical outcome than their functional activity ex vivo. This suggests that immunological antitumour mechanisms in vivo are not always related to generally accepted functional parameters of lymphocytes, such as cytotoxicity or cytokine production, ex vivo. The proliferative status of CD8$^+$ T lymphocytes seems promising for prediction of response in cancer immunotherapy.*

The effect of chemotherapy or so called 'targeted' antitumour therapy of the most common cancers is still quite modest, with a survival improvement of a few weeks or months (1). Increased understanding of the immune system and the increasing recognition of the importance of host-tumour interaction may lead to developing new immunotherapeutic strategies and improvements in therapeutic choices for patients with cancer (2).

Correspondence to: Professor D. Characiejus, Faculty of Medicine, Vilnius University, M.K.Čiurlionio 21, LT-03101 Vilnius, Lithuania. Tel: +370 52398728, Fax: +370 52469210, e-mail: dainius.characiejus@gmail.com

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Accumulating evidence demonstrates that the length of the survival of cancer patients may depend on the antitumour immune response of the host. In various tumour types, including lung, urothelial, renal cell, esophageal, colorectal, ovarian and endometrial cancer, and malignant pleural mesothelioma (3-11), the presence of CD8$^+$ T-cells within a tumour is correlated with longer survival. The presence of proliferating CD8$^+$ lymphocytes is important for the control of disease (3). Some studies suggest that quantities and proportions of T (mainly CD8$^+$) lymphocyte subsets in peripheral blood may also be related to survival of cancer patients (12-14). These data also show that antitumour immune responses in cancer patients are not uniform: in some patients, the immune system is capable of controlling the disease to a greater extent than in others.

Cancer immunotherapy is a rapidly expanding area of research and clinical practice. Cytokines, monoclonal antibodies, vaccines and adoptive cell immunotherapy are increasingly being studied in trials and used in clinical practice. Given the variable extent of antitumour immune reactivity in different patients, the question arises whether effectiveness of a given immunotherapeutic approach may depend on a pre-existing immune state of a patient.

Prognostic markers are baseline (pre-treatment) measurements that provide information about a patient's likely long-term outcome, either untreated or with standard treatment. Prognostic markers can be used to determine whether a patient requires any systemic treatment or any treatment beyond the standard treatment. Predictive markers are baseline measurements that indicate whether a patient is likely (or unlikely) to benefit from a specific drug or regimen (15). Molecular markers, gene expression profile in tumour cells and serum proteins may have a predictive value in cancer immunotherapy (16-19). Immunotherapy, by definition, involves immunological events. Therefore, it is logical to assume that immune parameters will eventually

yield the necessary predictive indicators (20). In a number of studies, the monitoring of immune parameter values during immunotherapy was performed. Post-treatment variables, such as the height of rebound lymphocytosis, treatment-induced thrombocytopenia, development of autoimmune thyroiditis and vitiligo, and changes in absolute number and frequency of peripheral T-regulatory cells were found to correlate with the outcome of immunotherapy (21). The aim of the present paper is to review the relationship between pre-treatment immune parameter values and the outcome of immunotherapy. In some studies, the predictive significance of immune parameter values cannot be strictly separated from their prognostic significance. Thus, in some cases, the prognostic significance of immune parameter values will be also mentioned.

Prediction of Response to Bacillus Calmette-Guérin Immunotherapy

Intravesical instillation of bacillus Calmette-Guérin (BCG) was the first immunotherapy to demonstrate reproducible activity in cancer patients (22) and is regarded to be the most effective immunotherapy identified to date. Recent analyses confirm the long-lasting effect of treatment with intravesical BCG on recurrence and progression in high-risk bladder cancer patients. This treatment now is considered also in cases of indermediate-risk bladder tumours (23). However, approximately 30-40% of patients fail to respond to BCG immunotherapy.

Evidence has been accumulating for some time that immunological markers are predictive of the BCG response. Conversion of purified protein derivative (PPD) skin reaction from negative to positive, formation of granulomas in the bladder, and an increase in levels of urinary cytokines, interleukin-2 (IL-2) in particular, during or after treatment have been correlated with response (20). However, changes of immune parameter values during or after BCG administration do not help in selecting patients for this type of treatment and cannot be regarded as predictive.

Some evidence indicates that immune parameter values measured before treatment can correlate with therapeutic effects of BCG. In a recent study, Ayari *et al*. (24) showed that maintenance BCG (more than one cycle) was highly effective in patients with a low level of $CD83^+$ tumour-infiltrating dendritic cells at time of resection, but BCG showed reduced efficacy in patients with a high level of $CD83^+$ tumour-infiltrating dendritic cells. In the same patient population, a strong infiltration of $CD68^+$ tumour-associated macrophages was associated with an increased risk of recurrence.

Thus, although studies on the relationship between pre-treatment immune parameter values and the outcome of BCG therapy of bladder carcinoma are relatively scarce, they suggest that the response to BCG therapy can be predicted.

Prediction of Response to Cytokine Therapy

Reproducible therapeutic activity of recombinant cytokines was demonstrated in some forms of cancer, including renal cell carcinoma and melanoma in the 1980s (25). Although the complete or partial response rate with cytokines is rather low, some long-lasting remissions are achieved (26-29). It is extremely important to find pre-treatment immune parameters that would predict prolongation of survival after cytokine therapy. Tumour-infiltrating immune cells and lymphocytes in peripheral blood have been studied in an attempt to find such predictive parameters.

Tumour-infiltrating immune cells. Håkansson *et al*. (30) observed a significantly longer time to progression and longer overall survival among metastatic melanoma patients with moderate to high numbers of $CD4^+$ tumour-infiltrating lymphocytes compared to patients with low numbers of these cells before initiation of chemoimmunotherapy (cisplatin, dacarbazine, interferon (IFN)-α2b). In 85 patients with metastatic renal cell carcinoma, Donskov and von der Maase identified the presence of intratumoural neutrophils (>0 cells/mm^2 tumour tissue), low intratumoural $CD57^+$ cell count (<50 cells/mm^2 tumour tissue), and high blood neutrophil count (>6.0×10^9/l) as independent poor prognostic factors for the outcome of IL-2-based immunotherapy (31). In a recent study, the same group established that long-term survival of IL-2-treated metastatic renal cell carcinoma patients was associated with low-baseline intratumoural $FOXP3^+$ cells and a modest absolute rise in these cells during treatment. Achieving high numbers (>180 cells/μl) of on-treatment $FOXP3^+$ intratumoural immune cells was associated with poor survival (32).

Increased tumour infiltration of mature ($CD83^+$) dendritic cells predicted longer survival of metastatic renal cell carcinoma patients who received cytokine-based immunotherapy (IFN-α monotherapy or a combination of IFN-α, IL-2 and fluoro-pyrimidine). Interestingly, expression of S100 protein on intratumoural dendritic cells was associated with tumour size reduction after therapy, but not with survival (33).

Leukocytes in peripheral blood. Fumagalli *et al*. (34) have found that the pre-treatment lymphocyte count in peripheral blood is a prognostic factor for overall survival in metastatic renal cancer patients treated with subcutaneous IL-2 immunotherapy. The prognostic/predictive significance of the peripheral blood lymphocyte count for overall survival was independent of tumour response and of major prognostic clinical characteristics (performance status, time from primary diagnosis and number of metastatic sites).

High pre-treatment counts of neutrophils and total leukocytes in blood were confirmed as independent prognostic factors for short overall survival in stage IV melanoma patients undergoing IL-2-based immunotherapy (35).

A panel of lymphocyte subsets ($CD3^+$, $CD19^+$, $CD16^+56^+$, $CD4^+$, $CD8^+$, $CD4^+CD45RO^{high}$ and $CD8^{high}CD57^+$) was analysed by this group in peripheral blood of 85 advanced renal cell carcinoma patients in an attempt to find immunological parameters with the predictive significance for survival after treatment with IFN-α2b (14). In addition to absolute counts, the percentage of $CD8^{high}CD57^+$ lymphocytes in the $CD8^+$ subset, the percentage of $CD4^+CD45RO^{high}$ lymphocytes in the $CD4^+$ subset and the $CD4^+/CD8^+$ ratio were analysed. The only lymphocyte subset that significantly correlated with survival of IFN-α2b treated advanced renal cell carcinoma patients was $CD8^{high}CD57^+$ lymphocytes. $CD8^{high}CD57^+$ lymphocytes can be considered to be T cells, whereas CD8lowCD57+ lymphocytes may contain NK cells (36, 37). Non-treated patients with ≥30% $CD8^{high}CD57^+$ lymphocytes in the $CD8^+$ subset had a shorter survival time than patients with <30% $CD8^{high}CD57^+$ lymphocytes in the $CD8^+$ subset. Thus, results of this study suggest a negative role for $CD8^{high}CD57^+$ lymphocytes in the natural course of advanced renal cell carcinoma. Treatment with IFN-α2b increased overall survival only in the subgroup of patients with ≥30% $CD8^{high}CD57^+$ lymphocytes. Unpublished observations from the current authors suggest that numbers of $CD8^{high}CD57^+$ lymphocytes decrease in these patients during therapy with IFN-α2b.

Remarkable differences in survival, depending on pre-treatment levels of $CD8^{high}CD57^+$ lymphocytes in peripheral blood, were observed by the current authors in 16 melanoma patients treated with adjuvant IFN-α2b (38). Patients with <23% $CD8^{high}CD57^+$ lymphocytes in the $CD8^+$ subset prior to treatment survived considerably longer than patients with >23% $CD8^{high}CD57^+$ lymphocytes. Lower pre-treatment values (<23%) of these lymphocytes tended to increase, whereas higher values (>23%) tended to decrease during the first few months of treatment with IFN-α2b. Thus, in contrast to a negative role of $CD8^{high}CD57^+$ lymphocytes in advanced renal cell carcinoma, these lymphocytes seem to play a positive role in adjuvant treatment with IFN-α2b of melanoma. These data are in line with other published reports suggesting that an increase in $CD8^+CD57^+$ T lymphocytes is associated with prolonged survival of melanoma patients after vaccination therapy (39, 40).

The predictive and/or prognostic significance of peripheral blood $CD8^{high}CD57^+$ lymphocytes was also shown in patients with non-muscle invasive bladder carcinoma receiving intravesical instillations of IL-2 after transurethral resection of tumours. High pre-treatment levels of $CD8^{high}CD57^+$ lymphocytes in peripheral blood independently predicted short recurrence-free interval in these patients (41).

The above data on cytokine therapy indicate that both tumour-infiltrating cells and peripheral blood lymphocytes may have a predictive value. Peripheral blood represents only about 2% of the total lymphocyte pool in the human body. Thus, on the one hand, it may seem unlikely that the lymphocytes of this small compartment are representative of the other 98% lymphocytes in all organs, and a malignant tumour. On the other hand, it has been estimated that roughly 500×10^9 lymphocytes travel through the blood each day. This is about the same number as the number of lymphocytes within the human body. Since most lymphoid and non-lymphoid organs are included in the migration/circulation routes of lymphocytes, alterations in the lymphocyte composition within these organs might be detected by studying lymphocyte subsets in the blood (42, 43).

Thus, there are reports suggesting a relationship between pre-treatment immune parameter values and the outcome of cytokine therapy. Unfortunately, in the majority of studies, the phenotype of immune cells with possible predictive value has not been determined (*e.g.* total leukocytes, total lymphocytes, total neutrophils), or has been determined by just one marker (*e.g.* $CD4^+$ lymphocytes, $CD57^+$ cells, $FOXP3^+$ cells). This is obviously insufficient for the precise determination of a cell type. In only a few studies were at least two markers used for determination of the phenotype of immune cells. These studies suggest that the immune cell types predicting the response in cytokine therapy of cancer are subsets of $CD8^+$ T lymphocytes (Table I).

Prediction of Response to Monoclonal Antibody Therapy

Monoclonal antibodies probably are the most widely used cancer immunotherapeutics at present (44). Monoclonal antibodies that act directly on tumour cells are 'targeted' against an antigen expressed on tumour cells. Unexpectedly, responses are achieved only in a proportion of patients whose tumours express an antigen, against which the monoclonal antibody is targeted (45-47). This indicates that better predictors of response to monoclonal antibody therapy are needed.

Polymorphisms of receptors for immunoglobulin G (FcγRs). It has been suggested that immune mechanisms, including antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) may be involved in the killing of tumour cells by monoclonal antibodies. In ADCC, the antibody binds to tumour cells and then is engaged by effector cells, such as NK cells or macrophages, *via* their FcγRs. FcγRIIIa (CD16) is expressed on both NK cells and macrophages, whereas FcγRIIa and FcγRIIb (CD32) are found only on macrophages (48). Based on the evidence that properties of FcγRIIIa depend on the *FCGR3A* gene dimorphism with either a phenylalanine (F) or a valine (V) at amino acid position 158, Cartron *et al*. (49) evaluated the relationship between *FCGR3A* genotype and response to rituximab in 49 patients with untreated non-Hodgkin

Table I. *Summary of data indicating predictive significance of pre-treatment immune parameters in cancer immunotherapy.*

Immunotherapy	Tumour type	Predictive factor	Tissue	Correlation with therapeutic effect	References
BCG	Bladder cancer	$CD83^+$ dendritic cells, $CD68^+$ macrophages	Tumour	(–)	(24)
Cytokines	Metastatic melanoma	$CD4^+$ lymphocytes	Tumour	(+)	(30)
		Total leukocytes, neutrophils	Peripheral blood	(–)	(35)
		$CD8^{high}CD57^+$ lymphocytes	Peripheral blood	(–)	(38)
	Metastatic renal cell carcinoma	Neutrophils, $CD57^+$ cells	Tumour, peripheral blood	(–) (+)	(31)
		$FOXP3^+$ cells	Tumour	(–)	(32)
		$CD83^+$ dendritic cells	Tumour	(+)	(33)
		Total lymphocytes	Peripheral blood	(+)	(34)
		$CD8^{high}CD57^+$ lymphocytes	Peripheral blood	(+)	(14)
Monoclonal antibodies	Non-Hodgkin's lymphoma, chronic lymphocytic leukaemia, follicular lymphoma, breast cancer, colorectal cancer	FcγRIIIa 158V/V and FcγRIIa 131H/H genotypes	Genomic DNA from tumour or peripheral blood	(+)	(48-51)
Vaccines	Metastatic cancer, various primary sites	$CD4^+CD25^{high}$ regulatory T lymphocytes	Peripheral blood	(–)	(60)
	Colorectal cancer	Plasma levels of IL-6	Peripheral blood	(–)	(61)
T-Cell transfer	Metastatic melanoma	$CD8^+CD27^+$ T-cells	Peripheral blood	(+)	(64)

(+), Positive correlation with therapeutic effect; (–), negative correlation with therapeutic effect.

follicular lymphoma. The objective response rates at two months and at one year were 100% and 90%, respectively, in *FCGR3A*-158 V homozygous patients compared with 67% and 51%, respectively, in *FCGR3A*-158 F carriers. Disappearance of the *BCL2-JH* gene rearrangement (used for monitoring of molecular response) was observed in both peripheral blood and marrow at one year in 5 out of 6 homozygous *FCGR3A*-158 V patients compared with 5 of 17 of *FCGR3A*-158 F carriers. The observation of Cartron *et al*. (49) was confirmed in the study of Weng and Levy (48) including 87 follicular lymphoma patients who received rituximab as second-line treatment. Furthermore, these authors determined the association between FcγRIIa 131 histidine (H)/arginine (R) polymorphism and rituximab response. Both FcγRIIIa 158 V/V and FcγRIIa 131 H/H genotypes were independently associated with the response rate and progression-free survival in patients with follicular lymphoma (46). However, neither FcγRIIIa nor FcγRIIa polymorphisms predicted response to rituximab in B-cell chronic lymphocytic leukaemia (45).

The significance FcγRIIIa and FcγRIIa polymorphisms was reported for other humanized IgG1 antibodies, namely trastuzumab and cetuximab. FcγRIIIa 158 V/V genotype (and to lower extent of FcγRIIa 131 H/H genotype) appeared to be predictive for objective response rate and progression-free survival in HER-2/*neu*-positive metastatic breast cancer patients treated with trastuzumab-based therapy. The ADCC analysis showed that peripheral blood mononuclear cells of FcγRIIIa 158 V/V genotype had a significantly higher trastuzumab-mediated cytotoxicity than cells harbouring 158 V/F and 158 F/F genotypes (50). Metastatic colorectal cancer patients with FcγRIIa 131 H/H and/or FcγIIIa-158 V/V genotypes had longer progression-free survival after treatment with cetuximab plus irinotecan than 131 R and 158 F carriers (5.5 *versus* 3.0 months) (51).

The results described above indicate that polymorphisms of FcγRs can predict the response to monoclonal antibody therapy. However, there are some data against the involvement of ADCC in the clinical effect of antitumour monoclonal antibodies. No correlation was found between the *in vitro* susceptibility of pre-treatment tumour cells to rituximab-mediated ADCC and the clinical outcome in patients with follicular lymphoma (48).

FcγRs are also important in antibody-mediated antigen uptake and cross-presentation of tumour antigens by dendritic cells to stimulate specific $CD4^+$ and $CD8^+$ T lymphocyte response (52, 53). $CD4^+$ and $CD8^+$ T lymphocytes are considered to be the key players in antitumour immune responses. Considering the potential influence of FcγR polymorphisms on the priming of cellular immunity, it cannot be excluded that the predictive value of FcγR phenotypes is independent of ADCC, but depends on cellular immunity. Indeed, some data suggest that T lymphocytes may be involved in the therapeutic effect of monoclonal antibodies. Decrease in regulatory T-cell numbers (54, 55), increase in Th17 cell numbers (55) in the

peripheral blood and augmentation of HER-2/*neu*-specific CD4 T-cell responses (56) have been reported in advanced breast cancer patients receiving trastuzumab therapy. Therefore, studies aimed at finding a relationship between pre-treatment T lymphocyte parameters and clinical outcome in monoclonal antibody therapy would be of interest.

Prediction of Response to Vaccination Therapy

Using cancer vaccines to harness the power of the cancer patient's own immune system is a highly attractive and innovative approach to cancer management (57). However, many cancer vaccines have shown little evidence of clinical antitumour effect so far. In contrast, dendritic cell-based vaccines are associated with a low (approximately 14%) but consistent clinical response rate (58). To date, most efforts have been directed toward the immune monitoring of antigen-specific T-cells within peripheral blood of vaccinated cancer patients. Indeed, induction of tetramer-specific T-cells in melanoma patients after vaccination correlates with progression-free survival (59). Studies relating the survival of vaccine-treated cancer patients with pre-treatment immune parameter values are scarce. In one study (60), patients with several advanced malignancies (various primary sites) were divided into three groups according to their survival time after an intravenous injection of tumour cell-pulsed monocyte-derived dendritic cells and activated lymphocytes. It was shown that the percentage of regulatory T-cells ($CD4^+CD25^{high}$) among total $CD4^+$ T-cells in peripheral blood before the therapy was significantly lower in long-survival patients than in the short-survival patients. In another study, 14 colorectal cancer patients were treated with dendritic cells loaded with lysate from a cloned and selected melanoma cell line enriched in expression of cancer/testis (MAGE) antigens. Eleven out of the fourteen colorectal cancer patients were considered MAGE-positive. Although no complete or partial responses were observed in this study, four of the fourteen patients achieved stable disease. Pre-treatment levels of plasma IL-6 were lower in patients with stable disease compared to patients with disease progression (61).

Studies on the relationship between pre-treatment immune parameter values and the outcome of vaccination therapy of cancer patients are scarce. However, the existing evidence suggests that pre-treatment immune parameters may predict a response to vaccination therapy. Further studies in this field are needed.

Prediction of Response to Adoptive Cell Immunotherapy

Probably the earliest form of adoptive T-cell immunotherapy has been allogeneic hematopoietic cell transplantation. The assumption that donor T-cells exert a graft-*versus*-tumour effect was corroborated by demonstration of therapeutic effects of donor lymphocyte infusions (DLI) (62). The greatest success with DLI has been seen in chronic myelogenous leukaemia, where durable remissions are achieved in 70% to 80% of patients. DLI are less successful in other haematological malignancies, including acute lymphocytic leukaemia, multiple myeloma, chronic lymphocytic leukaemia and indolent non-Hodgkin's lymphoma (63).

Tumour-infiltrating lymphocytes or peripheral lymphocytes repeatedly stimulated *in vitro* with autologous melanoma cells often demonstrate *in vitro* recognition of melanoma cells based on assays of lysis or cytokine secretion. The techniques have been developed to grow large numbers of anti tumour lymphocytes using tumor-infiltrating lymphocytes. It was found that lymphodepletion in metastatic melanoma patients prior to transfer of expanded autologous tumor-infiltrating lymphocytes results in relatively high objective response rates ranging between 49% and 72% (64). Possible explanations for the effect of lymphodepletion include elimination of autologous regulatory T-cells and elimination of competition for homeostatic cytokines, such as IL-7 and IL-15, that are vital for T-cell survival. Thus, levels of autologous regulatory T cells, IL-7 and/or IL-15 might predict a response to adoptive cell immunotherapy. However, the data to prove this are not yet available.

Regarding the transferred lymphocytes, the anti tumour response correlated with the mean telomere length of the cells infused and with the number of $CD8^+CD27^+$ T cells infused (64). Telomere length is related to the capacity for cell division, even though this relationship in lymphocytes may be rather complex (65). Expression of CD27 antigen correlates with resistance to apoptosis, IL-2 production, and proliferative potential of $CD8^+$ T lymphocytes (66). These data suggest that proliferative potential of the infused $CD8^+$ T lymphocytes can predict the success of the treatment.

Conclusions and Future Prospects

Prediction of response in cancer immunotherapy is promising for two main reasons. First, the success of immunotherapy may be increased by administering this type of treatment only to patients with biomarkers that correlate with positive therapeutic effects. Avoiding immunotherapy for patients with biomarkers that predict negative therapeutic effects becomes of utmost importance due to the possibility of the cancer-promoting effect of immunotherapy (67). Second, establishing the relationship between pre-treatment parameter values of the patient's immune system and the outcome of immunotherapy may help understand the therapeutic mechanisms induced by immune manipulation.

The evidence presented in this review strongly suggests that there is a relationship between pre-treatment immune

parameter values and outcome of immunotherapy of cancer patients. A summary of data indicating the predictive significance of pre-treatment immune parameter values in cancer immunotherapy is presented in Table I. The reviewed literature suggests that tumour-infiltrating cells of the immune system may have predictive value for immunotherapy, but probably they do not have an advantage over cells circulating in peripheral blood. Use of peripheral blood may be preferable due to the greater convenience of collection and analysis compared to using tumour-infiltrating cells.

The most important question to answer is, which of the immune parameter(s) is (are) the most promising for future studies of predictive biomarkers? Several lines of evidence suggest that subsets of $CD8^+$ T lymphocytes deserve special attention in search of predictive markers. $CD8^+$ T lymphocytes are well-documented effectors of tumour immunity (68). $CD8^+$, but not $CD4^+$, T-cells are required for the maintenance of tumour dormancy in an experimental mouse model (69). There is ample evidence for the prognostic significance of $CD8^+$ T lymphocytes in cancer patients (reviewed in the introduction). It is therefore logical to assume that numbers of $CD8^+$ lymphocytes may reflect antitumour immune reactivity of the cancer patient and predict the response to immunotherapy. Indeed, predictive significance of $CD8^+$ T lymphocytes has been demonstrated in cytokine therapy and in adoptive cell immunotherapy of cancer. Predictive significance of $CD8^+$ T lymphocytes in monoclonal antibody therapy, vaccination therapy, and in BCG immunotherapy still awaits investigation.

On the other hand, there is increasing evidence that markers on $CD8^+$ T lymphocytes may serve as measure of functional immune deficiency in patients with various diseases including cancer (70). Interestingly, the frequency of $CD8^+$ lymphocyte subsets ($CD8^+CD28^-$), but not of $CD4^+$ lymphocyte subsets, predicted the responsiveness to influenza vaccination in elderly individuals (71).

Most of the evidence reviewed in this article concerns the *in vivo* numbers of cells of the immune system. We were not able to find any published data on the relationship between pre-treatment functional characteristics of immune cells *ex vivo* and the outcome of immunotherapy. Antitumour immune mechanisms *in vivo* may be more complex than mechanisms detected by lymphocyte functional studies *ex vivo*, such as cytokine production or cytotoxicity. Indeed, a dichotomy between *in vitro* $CD8^+$ T cell responses and *in vivo* antitumour immunity of these cells has been shown in an experimental mouse model (72, 73). Furthermore, presence in tumour biopsies of cytotoxic T-lymphocytes, as determined by the percentage of $CD3^+$ lymphocytes with granzyme B expression, was unexpectedly found to correlate with reduced overall survival in patients suffering from Hodgkin's disease or non-Hodgkin's lymphomas (74).

Several reports suggest that expansion of $CD8^+$ lymphocytes (mainly as $CD8^+CD57^+$ subset) in cancer patients is a prerequisite for the successful control of the disease (12, 13, 39, 40). These reports underscore the predictive importance of the proliferative potential of $CD8^+$ lymphocytes. Indeed, the proliferative potential of the infused lymphocytes is critical to the success of adoptive cell immunotherapy (64). It is interesting to note that in adult humans, $CD8^+$ T-cells have a more than two fold faster doubling time than $CD4^+$ cells (75). Thus, markers of the replicative status of $CD8^+$ T lymphocytes, such as CD57 and/or CD27, seem promising in studies of predictive biomarkers in cancer immunotherapy. Hopefully, the results of these studies will allow identification of cancer patients who would benefit most from immunotherapy.

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