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Review

Relevance of the T cell receptor for immunotherapy of cancer

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Introduction

The demonstration that T cells with specific cytotoxic activity against autologous tumor are present in the peripheral blood or at the tumor site in patients with cancer suggests that T cells are capable of recognizing tumor-associated antigens (TAA) [3, 67, 80]. Recently, it has been established that T cells use the T cell receptor (TCR) for recognition of antigenic peptides, which are presented by the major histocompatibility complex (MHC) molecules expressed on antigen-presenting cells [24, 94]. Tumor-derived peptides are also recognized by tumor-specific T cells in the context of the MHC molecules [16, 34, 181]. The TCR expressed on T cells belongs to the immunoglobulin superfamily of cell surface molecules [24, 94]. In addition to TCR, the CD3 complex of accessory molecules plays an important role in T-cell-mediated recognition [48, 82].

In patients with cancer, specific cytolytic T cells (CTL) have been often derived from lymphocytic infiltrates present at the tumor site (tumor-infiltrating lymphocytes, TIL). Following activation with T-cell-activating cytokines, TIL proliferate in culture and acquire potent antitumor cytolytic properties [42, 65, 67, 99, 106, 136, 151]. Based on this observation, the hypothesis has been advanced that TIL infiltrate the tumor in response to TAA and thus are enriched in autotumor(AuTu)-specific T lymphocytes, at least some of which may be able to lyse AuTu. Recognition by such CTL of an appropriately presented antigenic peptide should result in proliferation and expansion of AuTu-reactive T cell clones in the tumor [63, 67, 174, 179]. Indeed, the presence of a large number of T cells in tumors has been correlated with a prognostically favorable outcome in some cases [18]. In addition to the tumor site, AuTu-reactive CTL have been found in peripheral blood [66, 183] or malignant ascites [66] of patients with cancer, indicating that a systemic response to the tumor may be present or that redistribution of CTL from the tumor to the periphery might occur. For example, Yasumura and colleagues recently have obtained evidence for the presence of memory T cells with specific cytotoxic activity against AuTu in the peripheral blood of a patient free of the tumor burden, whose tumor (squamous-cell carcinoma of the tongue) had been removed 2 years previously [183].

Studies performed in animals bearing established tumors indicated that activated, adoptively transferred TIL had therapeutic efficacy [26, 125, 141]. Based on these promising initial findings, clinical trials with human TIL were started in the mid 1980s. TIL were isolated from tumors, expanded in vitro in the presence of interleukin-2 (IL-2) for 6–8 weeks and retransfused to patients with metastatic disease [85, 126, 150]. Using TIL and IL-2 for treatment of patients with metastatic malignant melanoma, Rosenberg and coworkers reported remission rates of up to 40%, including some long-lasting responses [126, 150]. However, the preparation of TIL for therapeutic administration to patients with cancer is complex, and AuTu-specific CTL are not consistently obtained in therapeutic TIL cultures [1]. For these reasons, better characterization of AuTu-specific CTL and improvements of methods for their activation and culture have been emphasized recently. Specifically, it is desirable to outgrow AuTu-specific CTL from TIL more effectively and consistently [71] and to simplify complex technologies required for TIL expansion. Attempts have been also made to define the repertoire of the TCR genes used by TIL freshly isolated from tumors or cultured in the presence of IL-2 [64, 108, 174]. At the same time, other studies have been focusing on defining the target on tumor cells for the TCR, namely, the antigenic peptide presented to CTL in the groove of the class I MHC molecules [16, 145, 154, 160, 161].

In this brief review, we will attempt to summarize the current knowledge about a role of TCR in recognition of tumor-associated peptides and about cellular mechanisms

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induced by TCR-mediated antigen recognition on tumor cells. In addition, we will briefly evaluate future prospects for the use of T cells in the treatment of malignant diseases.

Immunotherapy of malignant tumors using cytotoxic lymphocytes

Many human progressive or metastatic cancers such as disseminated malignant melanoma or metastatic renal cell cancer are resistant to conventional therapies, including chemotherapy or radiotherapy. In these types of cancer, immunotherapy has been tried over the past 10 years and, although its success rate has been relatively modest, it remains a promising alternative to the conventional therapies [10, 112, 123, 124, 178]. Efforts to improve immunotherapy have been ongoing worldwide in hope of generating highly effective cytotoxic antitumor effector cells [71, 107], introducing novel cytokines, combining immuno- with chemotherapy, or facilitating the delivery of immunotherapeutic agents to patients with cancer [78, 130, 166]. Although high-dose IL-2 has been used for therapy as a bolus or continuous infusion in patients with melanoma or renal cell cancer, considerable toxicity and limited clinical efficacy have encouraged a search for alternative biological approaches [128]. Regimens involving systemic administration of IL-2 and interferon α (IFNα) appear to be among the more promising therapeutic combinations of cytokines [4, 11, 127]. In addition to individual cytokines or combinations of different cytokines, IL-2 in combination with various effector cells has been used for therapy of advanced cancers [85, 124–126, 128, 150, 178]. IL-2 has been found to activate and induce expansion of lymphocytes capable of destroying cancer cells both in vitro and in vivo [57, 61]. These IL-2-activated effector cells were first thought to be a newly discovered lymphocyte population, independent of T or natural killer (NK) cells, and they were named lymphokine-activated killer (LAK) cells by Grimm and collaborators [57, 58]. Since then, it has been realized that LAK cells consist of a mixture of various cytotoxic lymphocyte populations, mainly NK cells but also T cells, with distinct immune phenotypes [30, 41, 149, 169]. By now, fairly extensive clinical experience with LAK cells and IL-2 has accumulated [128]. Overall, the response rate has been approaching 30%, and, although no statistically significant difference in the proportion of responses has been observed between therapy with high-dose IL-2 alone or IL-2 with LAK cells, a greater tendency toward long-term complete responses has been observed in patients with melanoma treated with LAK cells and IL-2 [128].

Regarding immunotherapy with adoptively transferred cytotoxic T lymphocytes, the very first reports of treatment with TIL expanded in vitro and IL-2 in patients with metastatic melanoma were encouraging [126, 150]. However, a need for a unique expertise, high costs and the considerable manpower required for this type of therapy have restricted its application to a few specialized institutions. Furthermore, it remains to be determined whether the promising clinical results obtained in the initial trials with TIL can be reproduced or improved upon, and whether therapy with TIL and IL-2 is effective in treatment of cancers other than metastatic melanoma. It also remains uncertain if TIL, considered to be mixtures of activated T lymphocytes which might or might not be enriched in AuTu-specific effector cells, are substantially more effective therapeutically than LAK cells. The mechanism through which CTL recognize tumor cells expressing the relevant antigenic peptide in association with an appropriate class I MHC molecule [67, 179] is quite distinct from that used by activated non-specific T cells as well as NK cells, which recognize tumor cell targets regardless of MHC restriction [121]. Although the precise nature of such non-MHC-restricted recognition is not clear, it is likely that cellular adhesion molecules play an important role in the process of effector-cell/tumor-cell interactions, leading to effective signal transduction and subsequent release of cytotoxic granules capable of lysing the cell membrane of tumor cells [139, 189]. Cellular adhesion molecules are probably also important in CTL/tumor-cell interactions as accessory molecules, which stabilize contact and facilitate or even regulate signalling through quantitative or conformational alterations in β1 or β2 integrins on the surface of effector cells. While expression and utilization of the TCR-CD3 complex by CTL distinguishes this subset of effector cells from NK or LAK cells, the cytolytic pathways activated by all these effector cells appear to overlap to a considerable extent, and it remains uncertain whether CTL employ some unique mechanisms to eliminate tumor cells as compared to non-MHC-restricted T cells or LAK cells [13]. It is currently thought that CTL are more effective in eliminating tumor metastases than are non-MHC-restricted effector cells [13]. However, few direct comparison studies of in vivo antitumor efficacy of these two types of effector cells have been performed. In general, their antitumor effectiveness in vivo might depend not only on the ability to directly lyse tumor cells or inhibit their growth but also to produce various cytokines, extravasate and localize to the tumor or interfere with the tumor vasculature.

The T cell receptor

The T cell receptor (TCR) is a complex of several polypeptide chains expressed on the T cell surface (Fig. 1) and consisting of variant and invariant regions, which are functionally closely associated with each other and with CD3 peptides [8, 86–88, 94, 176]. About 95% of human peripheral blood T cells express the αβ heterodimer, comprised of constant (C) and variable (V) regions [8, 86–88, 94, 176]. The V region of this structure is involved in antigen recognition. Associated with the αβ heterodimer are the γ, δ and ε chains [163] of the CD3 and the more recently discovered ν and ζ chains [70]. These five invariant chains are most likely involved in the antigen binding and responsible for signal transduction [70, 72, 163, 176]. The γ, δ and ε chains of CD3 are usually expressed as ε-γ or ε-δ heterodimers [27, 176], and the ν and ζ chains as ν-ζ, or ζ-ζ dimers [6, 177]. The sequence analysis has revealed that the ν and ζ chains are differently spliced products of the same gene and, therefore, some authors refer to them as the ζ chain family [48, 176]. It is assumed that by expressing different combinations of those dimers of invariant chains,

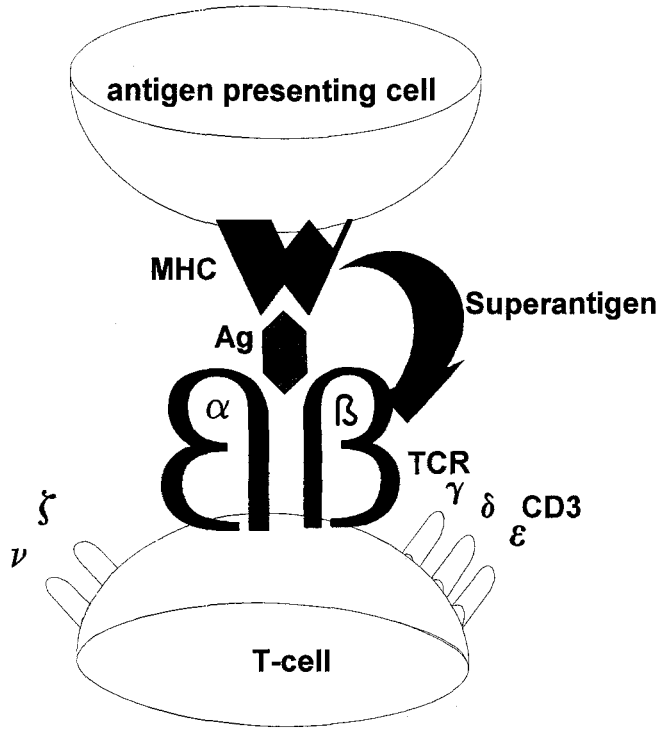

Fig. 1. T cell expressing the T cell receptor (*TCR*), including the associated molecules of the CD3 complex and its interaction with a conventional antigenic peptide or a superantigen bound to the MHC molecule expressed by an antigen-presenting cell

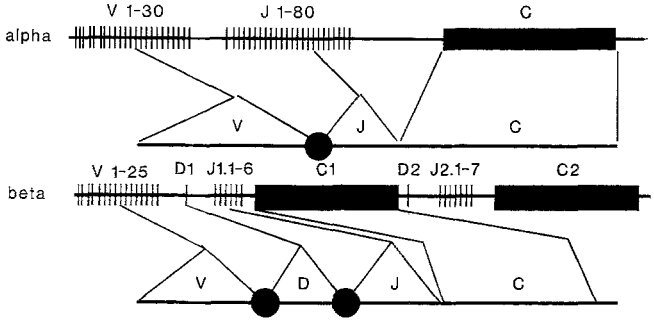

Fig. 2. Genomic organization of the T cell receptor (*TCR*) α and β chain genes and their rearrangement patterns that give rise to functional TCR genes in mature T cells ●, sites of N region diversity

the cell can be responsive to a variety of signals [82, 176]. The process of antigen recognition by the TCR involves the MHC molecules. With a few exceptions, antigens have to be presented by MHC molecules to induce T cell responses [14, 94, 152]. In order to be presented by MHC molecules, antigens first have to be processed to short (9–14 amino acids) antigenic peptides by antigen-presenting cells, e.g., macrophages. Subsequently, during a series of intracellular events, these antigenic peptides are fitted into a groove of the newly synthesized MHC molecules, and the resulting MHC-peptide complex is expressed on the surface of an antigen-presenting cell, as shown in Fig. 1 [56, 77, 155, 158]. The ability to recognize this MHC-peptide complex requires considerable diversity on the part of the TCR. The molecular basis for this diversity is provided by (a) the genomic organization of the genes for the variant chains of

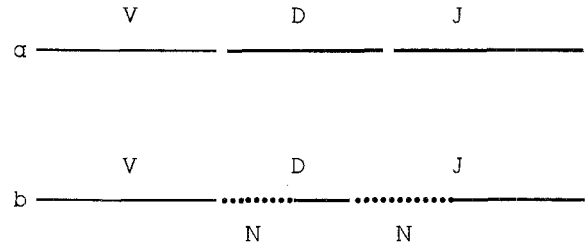

Fig. 3. The V, D and J elements of the CDR3 region of the TCR Vβ chain gene (*a*) and deletions of nucleotides at the D and J regions and addition of nucleotides at the sites of N region diversity (*b*)

the TCR and (b) complex rearrangement patterns of the defined fragments of these genes during early maturation of T cells [87, 94, 176] (Fig. 2). Thus, the gene for the TCR α chain consists of approximately 80 variable (V) region gene segments subgrouped in 30 V gene families (a V gene family is defined on the basis of more than 75% shared sequence homology) [87, 122], about 80 joining (J) regions [87], and one constant (C) region [138]. During the rearrangement of this gene, one of the V regions is connected to one of the J regions and to the C region. The product of this rearranged gene becomes a TCR α chain expressed on the mature T cell (Fig. 2) [87]. The unrearranged gene encoding the TCR β chain is somewhat more complicated than that encoding the α chain. Here, there are about 60 V gene segments subgrouped in 25 Vβ gene families [28, 38, 87], followed by one diversity (D) region (Cβ1), six J regions (Jβ1.1–1.6), one C region (Cβ1), another D region (Dβ2), a further group of seven J regions (Jβ2.1–2.7), and a second C region [79, 153, 185] (Fig. 2). The rearrangement of this gene proceeds according to the rules analogous to those for the α chain gene, resulting in a construct consisting of one V, one D, one J and one C region (Fig. 2) [2]. It is apparent that by multiplication of all the different TCR αβ gene segments, enormous variability of the final variant part of the TCR can be generated. This variability, however, is further increased by a phenomenon called "N region diversity", which involves the addition or deletion of nucleotides at all the junctional areas between the V and J regions of both chains. Figure 3 illustrates this phenomenon for the more complex TCR β chain [87].

The three-dimensional structure of the TCR remains to be discovered. However, performing comparative analyses of immunoglobulins and the TCR α and β chains, Choita and coworkers [24] have found that both types of molecule have similar structural features in the V regions. Like immunoglobulins, the TCR α and β chains appear to have three complementarity-determining regions (CDR) which presumably bind to the MHC as well as the antigenic peptide in the groove. CDR1 and CDR2 are coded by the V gene of the TCR. The CDR3 domain is located in the region of hypervariability. Therefore, it seems reasonable to postulate that if a T cell binds to an antigen-presenting cell, CDR3 binds to the most variable part of the MHC-peptide complex, namely the antigenic peptide. Currently, this appears to be the most acceptable hypothesis [15, 23]. Figure 3 illustrates the genetic basis for diversity of the CDR3 of the TCR β chain. During the rearrangement of fragments coding for the CDR3, several nucleotides are added next to

the already complete V region genes (the N region). Subsequently, one of the D regions, usually with deletions of some nucleotides on both ends, which sometimes are so extensive that the D region is no longer recognizable, binds to the 3′ end. Next, some more nucleotides are added, followed by the J region, which is usually missing one to three triplets at the 5′ end. This type of rearrangement allows for recognition by CDR3 of an enormous variety or peptides presented for the T cell use by MHC molecules on antigen-presenting cells [8].

The γ/δ TCR, which is also a heterodimer consisting of constant and variable regions and expressed in association with the CD3 complex on 3%–5% of human peripheral T cells, has a smaller repertoire of V regions compared with the αβ TCR [17, 87, 129]. Similarly to the αβ TCR, the genes for the γ/δ TCR are rearranged from the germline configuration, including the same gene segments described above [87, 142]. The unrearranged human TCR γ chain gene is comprised (from the 5′ to 3′ end) of four Vγ chain groups consisting of 11 V regions (Vγ1.1–8, Vγ2–4), four of which are pseudogenes; three J regions (Jγ1.1–3) one Cγ1, two further J regions (Jγ2.1, 2) and one Cγ2 [64, 91]. The rearrangement of this gene results in a V-J-C gene, including N region diversity at the VJ junction [17]. For the δ chain, eight V, two D, two J and one C regions have been identified [79]. Rearrangements can result in a V-N-(D-N-D-N)-J-C complex [38]. However, the importance of the γ/δ TCR for tumor immunology remains speculative, and we refer the reader to several recent reviews for a more detailed description of its genomic organization [17, 47, 87, 129, 142].

T cell activation pathways involving TCR/antigen interactions

Because of its unique structure, the TCR is responsible for recognition of peptides presented in the MHC groove and is capable of discriminating one peptide from another. However, the signal delivery via TCR/peptide interaction is not sufficient to induce proliferation and clonal expansion of the peptide-specific T cells, although it is apparently sufficient to induce expression of mRNA for interleukin-2 (IL-2) in the responding T cells [187]. To sustain the TCR-mediated activation signal, another signal, probably mediated by various members of the family of cellular adhesion molecules expressed on the T cell surface, is necessary [55, 131, 163]. When a ligand recognized by the cellular adhesion molecule, expressed on T cells is co-presented by an antigen-presenting cell, signal transduction is likely to occur. Among the cellular adhesion molecules known to participate in T cell activation are CD28 [154], CD11a/CD18 [162], and CD2 [84] and their respective ligands B7, ICAM-1, LFA-3 and certain very late antigens in the β_1 integrin family [33, 135]. The possibility that cellular adhesion molecules serve as accessory molecules or as signal receptors capable of initiating an alternative activation pathway is of special interest for tumor immunology. It has been suggested that antigen presentation and/or signal transduction via the TCR may be defective in tumor-bearing hosts [81, 97, 120]. Indeed, the inability of tumor-bearing hosts to mount protective immune responses against tumors has been thought to be due to ineffective antigen or peptide presentation by antigen-presenting cells. In some cases, tumor cells lack or express few MHC molecules, and thus do not effectively present antigens to T cells. Tumor-associated macrophages are known to be functionally deficient and may not be effective as antigen-presenting cells [93]. In view of these and possibly other defects in antigen presentation, activation of antitumor effector cells via cellular adhesion molecules or other surface molecules via alternative activation pathways is a potentially important mechanism. It is also likely that tumor cells themselves express cellular adhesion molecules or their receptors and thus are capable of inducing activation, albeit not MHC-restricted activation, of immune effector cells. Data are available in support of this hypothesis, indicating that susceptibility of tumor cells to AuTu-specific or non-specific effector cells may depend on the level of expression of ICAM-1 and other adhesion molecules on tumor cells [25, 101]. Little is known at present about expression by various tumors of the ligands able to mediate accessory or alternative activation of T lymphocytes, but there are indications that the CD28-B7 activation pathway is operating normally in the tumor microenvironment [188].

Activation of T cells after MHC-restricted recognition of peptides as well as cellular adhesion molecule ligands is coordinated by a cascade of enzymatic reactions referred to as "signal transduction" [72, 105]. Although our knowledge about various signal transduction pathways has been increasing rapidly in recent years, exact intracellular mechanisms operative between the initial recognition, subsequent signal transduction and final functional responses of T cells remain largely unknown. Nevertheless, functional differentiation of the T cell after recognition via the TCR of antigenic peptides or cellular adhesion molecule ligands, including its repertoire of cytokines or the ability to mediate cytolytic activity, may, in part, be programmed by the way molecules involved in signal transduction are grouped on the cell surface. Thus, for example, it has become evident that co-expression of one or another dimer of chains associated with the TCR αβ complex may ultimately lead to different functional properties of the T cell [72, 82]. After an MHC-restricted contact of the TCR with a peptide on the antigen-presenting cell, intracellular levels of a group of enzymes, including phospholipase C, protein tyrosine kinases, protein tyrosine phosphatases or protein serine kinases, which induce activation of T cell metabolism, increase within seconds [73, 105]. This observation implies that these enzymes are participants in the T cell activation. Furthermore, activation of these enzymes appears to be required for transcription of cytokine genes after TCR-mediated signaling. For example, activation of protein kinase C by phorbol esters has been shown to be necessary for cytokine production by T cells responding to the TCR- and CD28-mediated signals [147].

The cascade of signal transduction events in activated T cells is followed by differentiation into effector cells having activities important for the control of tumor growth and progression. Clonal proliferation of CTL in response to a tumor-associated peptide as well as secretion of various

cytokines and cytotoxic enzymes is instrumental for the development of effective antitumor responses. Some of the cytokines produced, such as tumor necrosis factor α (TNFα), interferon γ (IFNγ) or interleukin-6 (IL-6) are known to have direct antitumor effects [5, 54, 59, 102, 170]. Other cytokines, e.g., IL-2, IL-4, IL-7 and, possibly, IL-13, are capable of activating cytotoxic mononuclear cells [63, 96, 100, 143, 179]. Furthermore, there is increasing evidence for the ability of IL-2 to down-regulate growth of some tumors via functional IL-2 receptors expressed on tumor cells [164, 173]. IL-2 and a number of other cytokines mentioned above have been increasingly frequently used for immunotherapy of malignant human tumors in the past 10 years [11, 85, 112, 123, 124, 126, 127, 150, 166, 178]. The ability of activated T cells to secrete cytolytic molecules such as perforin/cytolysin or serine esterases/granzymes seems to be another specific function of T cells that have recognized an antigenic peptide [12, 35, 189]. These molecules enable the activated T cells to destroy tumor cells by forming pores in the tumor cell membranes with a subsequent loss of viability [12, 189].

Another pathway of T cell activation, which most likely does not play a major role in human tumor immunology, but should at least be mentioned briefly, is the TCR interaction with the so called "superantigens". Superantigens are generally products of bacteria (i.e., staphylococcal enterotoxins [46, 60, 75] or viruses, i.e., mouse mammary tumor viruses [37, 49], which are exceptionally potent T-cell-activating molecules. They are recognized by the TCR V regions irrespective of the hypervariable CDR3 region (see above) [23, 116]. Often they react with all T cells expressing a particular TCR Vβ family, leading to activation of the entire population of these T cells [46]. Recognition of a superantigen by immature T cells leads to clonal elimination in the thymus of all T cells bearing the Vβ family genes involved in the process of recognition [182]. In contrast, mature T cells respond to superantigens by a rapid activation followed by proliferation and cytokine production [140]. After superantigen-mediated activation, the responding T cells frequently undergo apoptosis, resulting in the elimination of all T cells expressing a particular TCR V beta family (for review see [20, 36, 45]).

Tumor-specific cytotoxic T cells

As indicated above, MHC-restricted T cells capable of mediating lysis of AuTu cells recognize processed antigenic peptides bound within the cleft of appropriate HLA molecules. Such AuTu-specific CTL have been demonstrated to be present in tumor-bearing hosts [26, 141] and in patients with cancer [3, 42, 63, 65, 67, 80, 99, 136, 151, 179]. TIL are thought to be enriched in this population of effector T cells [63, 174, 179]. However, TIL freshly isolated from human tumors generally fail to lyse AuTu cells [63, 95, 180] and either do not proliferate or proliferate poorly in response to AuTu, phytohemagglutinin, phorbol myristate acetate or other T-cell-activating agents [95, 180]. These same cells, however, activated with T-cell-stimulating cytokines such as IL-2, become responsive, i.e., proliferate and acquire the ability to lyse AuTu cells [3, 42, 63, 65, 67, 80, 99, 106, 136, 151, 179]. This in vitro phenomenon, indicating that CTL precursors are present among TIL, has been observed with a variety of tumors, including malignant melanoma [3, 67, 80], squamous-cell carcinoma [66], ovarian cancer [64, 65], gastric cancer [136], renal cell cancer [42, 83], glioma [99] and others [106, 133]. In some cases, CTL with specific AuTu cytotoxicity have been also derived from peripheral blood lymphocytes (PBL) obtained from patients with cancer [16, 18, 26, 42, 48, 63, 65, 66, 82, 99, 106, 136, 141, 151, 174, 179, 181, 183]. The specific AuTu reactivity of these cytolytic effector cells can be blocked by monoclonal antibodies (mAb) against the TCR and MHC molecules, strongly suggesting that the recognition of AuTu cells is both TCR-mediated and MHC-restricted [66, 67, 136, 181]. These studies support the view that, although T cells infiltrating the tumor or found in the circulation of patients with cancer may be unable to mediate effective AuTu responses, CTL precursors are present and are responsive to some exogenous activation signals. While fresh TIL often lack the ability to lyse AuTu cells, TIL isolated from a variety of human tumors and cultured in the presence of IL-2 and AuTu cells can develop into excellent antitumor effector cells [66, 67, 136]. On the basis of this type of evidence, it has been concluded that CTL reactive with AuTu are functionally suppressed in cancer patients [180]. Poor expression of T-cell-mediated antitumor activity in cancer patients with advanced malignancies may be related to the presence of tumor-derived immunosuppressive factors in the tumor environment as well as in the peripheral circulation. For example, there is evidence that transforming growth factor β (TGFβ), known to be produced by several human tumor types and to suppress T cell activation, might be, in part, responsible for blocking CTL in some patients with cancer [63, 118]. Recent studies in tumor-bearing mice have indicated that tumor-derived factors, probably different from TGFβ, are responsible for the failure of signal transduction in T cells obtained from these animals. Apparently, the TCR-CD3 complex on the surface of T cells isolated from mice bearing long-established tumors is unable to transduce activation signals effectively, signals that normally lead to the generation of antitumor effector cells [97]. More recently, the same deficiency has been demonstrated in TIL obtained from human solid tumors [43, 107]. On the other hand, TIL in situ have been shown to express activation markers, the MHC class II molecules or IL-2 receptors [63, 179] and mRNA for several cytokines [167], and produce cytokines in response to autologous tumors [133]. These data suggest that not all activation pathways and not all functions of TIL are blocked. Rather, selective inhibition of some activation pathways in T cells responsive to the tumor may occur in tumor-bearing hosts. From the point of view of immunotherapy, it is fortunate that this tumor-induced immunosuppression can be reversed by the use of exogenous cytokines or other activating agents.

Numerous studies of phenotypic and functional characteristics of TIL or PBL obtained from cancer patients have been performed after their in vitro activation with T-cell-stimulating agents in the search for AuTu-specific T cells [3, 16, 34, 48, 67, 80, 82, 106, 174, 179, 181]. In-

itially, in vitro activation of AuTu-specific CTL was attempted using relatively high doses of IL-2 (6000 IU/ml) [151]. Under these conditions, T cells cytotoxic against AuTu tumor cells could be generated, but CTL lines growing in high-dose IL-2 frequently have a broad spectrum of cytotoxicity and, by definition, should be categorized as LAK cells. More recently, lower doses of IL-2 (100–600 IU/ml) have been used to generate CTL lines capable of lysing AuTu tumor cells. This approach seems to result more consistently in expansion of the MHC-restricted AuTu-specific CTL, which demonstrate high levels of cytotoxicity against AuTu, may also kill allogeneic histologically related tumor cells but do not lyse allogeneic unrelated tumors [66, 136]. In some cases, e.g., using lymph node lymphocytes of patients with pancreatic cancer, it has been possible to generate non-MHC-restricted AuTu-specific CTL lines consistently in vitro, which recognize tumor-associated mucins [7, 68, 69]. These non-MHC-restricted mucin-specific CTL have been studied by Finn and collaborators, and they have been demonstrated to recognize peptides in the polar peptide mucin core composed of repeating units nine amino acids long [7, 68, 69]. Evidently, tumor-associated mucins composed of underglycosylated repeating subunits are immunogenic and are easily recognized by CTL without a need for antigen presentation on HLA molecules. Thus, not all tumor peptides require antigen presentation in the groove of the MHC molecule.

In some cases, it has been shown that addition of cytokines other than IL-2, e.g., IL-4 or IL-7 or even IL-13, may further facilitate generation of AuTu-specific CTL in vitro [96, 100, 107]. To maintain the specificity of these CTL lines, culture with fresh or irradiated AuTu cells seems to be required, presumably because expansion of these CTL in vitro is dependent on the activation signal involving TCR/tumor-peptide interactions. Schwartzentruber and coworkers [132, 133] have shown that stimulation of specific CTL with AuTu but not unrelated allogeneic tumor cells leads to secretion of cytokines, possibly in response to the MHC-restricted recognition of peptides derived from TAA. Other groups reported the release of cytotoxic granules from T cells in response to AuTu cells added to T cell cultures [35].

To obtain more detailed information about tumor-specific CTL, several groups have successfully attempted to clone by limiting dilution and subsequently study phenotypic and functional features of these cells [16, 65–67, 82, 136, 174, 179, 181]. Most of the initial cloning analyses of AuTu-specific T cells were performed in patients with malignant melanoma. Clones of AuTu-specific T cells obtained from PBL or TIL of such patients and successfully maintained in culture were used to study effector-cell/tumor interactions [34, 67, 161, 181]. Boon and colleagues have utilized T cell clones as probes for defining the nature of antigens recognized by the T cell clones on AuTu cells [154, 160, 161]. The family of shared melanoma-associated antigens named MAGE, which are recognized by T cells as nine-amino-acid peptides presented by HLA-A1 molecules, has been defined by these investigators [154, 160, 161]. Others have also provided evidence for the presence of both unique and shared antigens on melanoma [31, 181, 186], some of which are presented to $CD8^+$ T cells by HLA-A2 molecules [62, 76, 181]. Furthermore, melanoma cells that failed to express HLA-A2 were shown to be resistant to lysis by autologous CTL and were killed only when transfected with the HLA-A2 gene [62, 76]. In addition to HLA-A1 or HLA-A2, other class I molecules have been shown to present TAA-derived peptides. Wölfel and coworkers demonstrated that the same melanoma antigen could be presented by HLA-A2 and HLA-Bw6 molecules [181]. In a study performed by Yasumura and colleagues [184], tumor-specific CTL derived from PBL of a patient with squamous-cell cancer of the tongue were found to have a cytolytic response restricted by HLA-A2 and HLA-B44 molecules. The data from this study indicated that T cells expressing the TCR Vβ6 gene recognized an antigen epitope presented by HLA-A2 molecules, while an antigenic epitope presented by HLA-B44 molecules was recognized by autologous T cells expressing the TCR Vβ2 gene.

Recent data also suggest the existence of shared tumor antigens on tumors with different histologies. Studies by Barnd et al. [7] and Jerome and coworkers [68] indicate that CTL recognize tandem repeats of the mucin polypeptide core, which can be expressed by breast, pancreatic, ovarian and perhaps other epithelial cancers [7, 29, 68, 186]. As indicated above, this type of recognition is not restricted by MHC molecules, but is mediated by TCR, and it represents the only exception to the TCR-peptide-MHC activation complex discovered so far [7, 68].

Expression of TCR V genes in cytotoxic T cells

At the site of T cell infiltration of a tumor, clones of activated T cells responding to TAA are expected to be present. Therefore, the hypothesis has been that in tumors infiltrated by T cells, TCR gene expression may show either a monoclonal or oligoclonal pattern [108, 174]. The presence of restrictions in expression of the TCR V genes could be taken as evidence of clonal expansion of T cells responsive to the antigen driving the local immune response. Monoclonal antibodies against V regions of the TCR have been used to document such restricted cellular responses [19, 114]. However, only a limited number of such mAbs are available for human V chains. As an alternative, several qualitative or semi-quantitative approaches utilizing the polymerase chain reaction (PCR) have been introduced to test the hypothesis. Both approaches are based on a similar principle. RNA is extracted from the circulating T cells or tissues infiltrated with T cells and reverse-transcribed (RT) into cDNA. This cDNA is then amplified by PCR using a 3′ primer annealing in the C region of a given TCR chain gene and 5′ primers, which recognize the different V region gene families of the TCR α or β chains. By introducing internal standards and performing PCR in the presence of radioactive isotopes, the percentage of gene expression of each Vα or Vβ region can be determined [22, 38, 122, 174]. Using RT-PCR, the oligoclonal T cell repertoire at the site of disease has been found in several autoimmune diseases associated with T cell infiltrations including rheumatoid arthritis [111], autoimmune thyroiditis [32] or multiple

sclerosis [110]. More recently, evidence has been obtained for oligoclonal T cell receptor expression in tumors infiltrated by T cells [21, 39, 40, 44, 74, 108, 109, 113, 115, 134, 137, 146, 148, 159, 165, 171, 174, 175]. In 1990, Nitta and coworkers were the first to report the predominant expression of Vα7 among TIL in seven out of eight T cell infiltrates in uveal melanomas in situ [108]. They concluded that a shared melanoma antigen might have been the initial stimulus for induction of proliferation of T cells expressing Vα7 [108]. In later publications, the same group of investigators described restricted TCR Vα or Vβ expression in other melanomas, medulloblastomas and gliomas [109]. However, these studies were performed with a non-quantitative PCR system and, therefore, interpretation of these results has been equivocal. More recent work, using quantitative RT-PCR to analyze the TCR repertoire of TIL in situ, has confirmed the oligoclonality of T cells infiltrating a variety of human tumors, including malignant melanoma [148, 171, 172], hepatocellular carcinoma [174], basal cell carcinoma [146], renal cell cancer [21], ovarian cancer [44, 107] and neuroblastoma [159]. While these observations have suggested that oligoclonal expression of certain TCR Vα or Vβ genes by T cells accumulating at the tumor site or even by T cells in the circulation of patients with cancer may be related to an immune response directed at the AuTu-related antigen or peptide, preferential expression of a particular TCR V region gene in response to one tumor type has not been confirmed.

Regarding the studies of TIL in malignant melanoma, a restricted repertoire of the TCR V genes has been described, but the restriction patterns were different in each patient within the patient populations studied [148, 171, 172]. These results may suggest that TIL respond to different melanoma antigens expressed on tumors obtained from different patients but might, in part, also be explained by presentation of the same peptide by different restriction elements of the MHC molecules. In contrast, in a metastasis of a disseminated malignant melanoma, Ferradini and colleagues have identified completely diverse hypervariable regions within the same TCR Vβ region genes and have suggested that non-specific, inflammatory-like infiltration with T cells occurs in advanced tumor stages [39]. On the other hand, the same group of investigators reported later that, through direct sequencing of TCR transcripts, it was possible to show that unique T cells were selected and amplified at the tumor site in a spontaneously regressing melanoma lesion [40, 92]. In hepatocellular carcinoma, we showed that the frequency of gene expression for a certain Vβ region was as high as 32% in TIL or PBL examined by RT-PCR for expression of 20 Vβ gene families [174]. In peripheral blood obtained from healthy donors and analyzed using the same technique, the percentage of Vβ gene expression ranged from 0 to 14% and all 20 Vβ gene families were represented [172, 174]. Sequencing analyses of the amplified products of the two most highly expressed Vβ families in hepatocellular carcinoma demonstrated sequence homology in the majority of the clones that were sequenced, suggesting that a clonal proliferation, possibly in response to a TAA, had occurred in vivo [174]. However, the possibility has to be considered that preferential expansion of T cells with the same TCR Vβ gene restriction in PBL and TIL obtained from the same patients with hepatocellular carcinoma might indicate a generalized response to an antigen from another source, e.g., a viral antigen [174]. In further studies of Vβ gene expression in TIL, cells isolated from malignant melanoma and those obtained from ascites to patients with ovarian carcinoma were analyzed after in vitro activation and culture in the presence of cytokines [64, 172]. In melanoma TIL, cultured for therapeutic purposes in the presence of IL-2 and IL-4 but without addition of irradiated AuTu cells, Vβ gene expression did not correlate with either the predominant Vβ expression before culture or with AuTu cytotoxicity of cultured effector cells [172]. These studies suggest that, in cultures without added AuTu cells, selection of T cells that find optimal culture conditions occurs and that these cells outgrow AuTu-specific T cells [171, 172]. Ioannides and Freedman [64] observed preferential usage of Vβ8.1 and Vβ6.7 in CTL lines established from malignant ascites in five patients with ovarian cancer and suggested the possible association between expression of certain TCR Vβ genes and the ability of T cells to mediate AuTu cytotoxicity. Our own studies, with CTL specific for squamous-cell carcinoma of the head and neck, support this hypothesis. Overall, these studies further strengthen the argument in favor of the role of clonally restricted cytolytic T cells in human AuTu responses.

Several recent studies of TCR V genes in cancer patients who have received tumor vaccinations indicate that oligoclonal restrictions in the use of these genes are detectable [74, 175]. In a single case of a patient with bilateral renal cell cancer and two lung metastases, we showed a predominant expression of the TCR Vβ13.1 gene in a lung nodule that responded to treatment with irradiated AuTu cells and in vitro tumor-sensitized lymphocytes obtained from a vaccine-draining lymph node [175]. This lung nodule contained prominent T cell lymphocytic infiltrate [175] and T cells cultured from the lesion in the presence of IL-2 and irradiated AuTu cells have been shown to be $CD4^+$ and able to kill AuTu preferentially in a ^{51}Cr-release assay. Although in non-responding renal tumor, Vβ13.1 gene expression was also somewhat increased in comparison to PBL, this tumor was only moderately infiltrated by cells which, upon expression with IL-2 and AuTu, gave rise to a mixed population of $CD3^+CD56^-$ T cells and $CD3^-CD56^+$ NK cells [175].

The studies of TCR V gene expression in fresh or cultured TIL that have been performed to date are summarized in Table 1. Altogether, the available data strongly support the hypothesis that AuTu-specific T cells recognizing TAA or peptides via the TCR are present in the tumor and blood of patients with cancer and that these T cells may play an important role in response against malignancies. Possible consequences of these findings for tumor immunotherapy are discussed below.

Future prospects for immunotherapy with tumor-specific T cells

As a result of a greatly improved understanding of interactions between AuTu-specific effector T cells and tumor cells via the TCR-peptide-MHC complex, a large number

Table 1. T cell receptor Vα or Vβ gene expression in fresh lymphocytes and in lymphocytes activated in vitro, all derived from mononuclear cell infiltrates in human solid tumors or from peripheral blood lymphocytes of patients with cancer

Tumor type	Cell type	TCR restriction	Reference
Uveal melanoma	TIL in situ	Vα7	[31]
Malignant melanoma	TIL in situ	Vα, Vβ oligoclonal[a]	[155]
	TIL in situ	Vβ1, 3, 7, 10, 13, 14	[157]
	TIL in situ, primary lesions	Vα4, Vα22, Vβ8	[158]
	Metastasis responding to IL-2	Vβ4, 13, 14, 16	[169]
	Metastasis responding to IL-2	Vβ13.1, Jβ1.1	[170]
	Metastasis	No restriction	[163]
	Responding to IL-2	Vβ, Jβ, VDJ oligoclonal[a]	[166]
	In vivo primed T cell	Vα, Vβ	[164]
	Cultured TIL	Vα17, Vβ7	[156]
	CTL clones and TIL in situ	Vα8.2, Vβ2.1	[167]
Ovarian cancer	Tumor-specific CTL	Vβ5, 6, 8	[32]
	Cultured TIL	Vβ oligoclonal[a]	[161]
	Tumor-specific CTL	Vβ2, 6, 5.1	[168]
Hepatocellular carcinoma	TIL in situ	Vβ oligoclonal[a]	[19]
Basal cell cancer	TIL in situ	Vβ1, 2, 5.1, 6, 8	[159]
Renal cell cancer	TIL in situ	Vβ oligoclonal[a]	[160]
	Metastasis responding to IVS	Vβ13.1	[165]
Neuroblastoma	TIL in situ	Vα, Vβ oligoclonal[a]	[162]
Squamous-cell cancer	Tumor-specific CTL clones	Vβ6 or Vβ2	[147]
Adrenal cell cancer	Cultured TIL	Vβ6 or Vβ8	[188]

TIL, tumor-infiltrating lymphocytes; CTL, cytotoxic T lymphocytes; TCR, T cell receptor; IVS, in vitro sensitization

[a] Vα or Vβ repertoire was restricted compared to autologous or normal peripheral blood T cells, but the restrictions were different in TIL obtained from various tumors of the same histological type

of novel approaches to activation, generation, and in vitro expansion of tumor-specific CTL have been proposed. These include in vitro activation of effector cells with combinations of cytokines or mAbs, the addition of irradiated tumor cells or tumor-derived peptides to cultures of effector cells, and new gene-transfer technologies [104]. Strategies for in vivo activation of antitumor immune responses using cytokines, tumor cells transduced with cytokine genes or tumor vaccines have also been evaluated [52, 53, 81, 107, 144, 161]. Since a detailed description of all these approaches to immunotherapy is beyond the scope of this review, we prefer to focus on a few most promising strategies.

In a number of studies, various combinations of T-cell-activating agents have been utilized to increase activity and preferentially induce outgrowth of tumor-specific T cells in culture. This has proven to be a difficult and often unrewarding task, because cytokines are not capable of T cell activation in a specific manner [55, 187]. Cytokines can and do promote growth of tumor-specific CTL following their activation via the TCR and other T-cell-associated molecules [163]. Therefore, effective presentation to the tumor-specific T cell of the relevant TAA or peptide is initially required, and contact at carefully timed intervals with tumor cells seems to be necessary to proliferate T cells able to maintain specific AuTu cytotoxicity in long-term cultures [171, 172]. While this strategy appears to be feasible, it does require fresh or cultured AuTu, HLA typing, information about expression of a given TAA by the patient's tumor and selection of effective antigen-presenting cells. However, human tumor cells in numbers sufficient to stimulate therapeutic CTL cultures repeatedly during the course of expansion are seldom available. Since increasing numbers of peptides derived from TAA are being identified and are expected to be soon available as purified or synthetic products, it might be possible in the future to facilitate culture of AuTu-specific T cells by adding peptides to their cultures. Of special interest in this context are antigens expressed by a number of different tumors such as tumor-associated mucins [7, 68, 69] or other TAA shared within the same tumor type [154, 160, 161]. Such peptides could be synthesized biochemically and added to therapeutic T cell cultures in the presence of antigen-presenting cells expressing the MHC molecules able to present the peptide to the TCR. Following such in vitro sensitization, AuTu-specific cytotoxic T cells obtained from the patient's PBL, TIL or lymphnode lymphocytes (LNL) could be adoptively transferred to the donor in conjunction with IL-2 or other cytokines. Therapeutic efficacy of the adoptively transferred CTL would, in theory, depend on their ability to localize to the tumor or its metastases and to kill AuTu cells. However, it remains unconfirmed that human CTL are localized to the tumor in any substantial numbers or that their antitumor efficacy in vivo is dependent on cytolytic activity.

In vitro activation of T cells to induce or up-regulate specific lytic activity against AuTu, as described above, involves substantial effort and expense, and its therapeutic effectiveness is unproven at this time. A reasonable alternative might be vaccination of patients with TAA, using

peptides derived from AuTu cells or synthetic peptides [9, 81, 98, 144]. This principle of in vivo activation of T cells has been discussed for many years, but most of the clinical trials performed so far have not been promising [144]. This could be explained by the lack of complete understanding of antitumor responses, resulting in imperfect vaccines or ineffective administration of vaccines that could work otherwise. In view of substantial recent advances in the characterization and synthesis of tumor peptides and in our understanding of the mechanisms of presentation and recognition of these peptides, it appears likely that current vaccination efforts might be more successful. One strategy for in vivo vaccination of cancer patients involves administration of synthetic peptides or peptides derived from TAA. Although theoretically attractive, no effective tumor peptide vaccine is available at this time. Furthermore, not only the nature of tumor peptides, but also their immunogeneity in tumor-bearing hosts, the optimal route of administration, and the involvement of appropriate antigen-presenting cells would have to be evaluated before this strategy can be clinically applied. At least one synthetic vaccine, containing mucin peptides, is being currently evaluated for safety and ability to induce delayed-type hypersensitivity in patients with cancer [91].

Another promising new technology, already in use in phase I clinical trials, employs genetically engineered tumor cells transduced with cytokine genes. Administration of tumor cells transduced with IFNγ and/or IL-2 genes as tumor vaccines in animal models of tumor metastasis produced encouraging results [50, 51, 103, 117, 168]. This type of vaccination is based on the hypothesis that continuous production of IFNγ by transduced tumor cells may lead to a cytokine-mediated increase of MHC expression and thus better antigen presentation by the tumor or antigen-presenting cells. Additionally, the production of IL-2 by tumor cells should lead to further activation and promotion of AuTu-specific CTL at the site of vaccination [50, 156, 157, 168]. The strategy of improving the presentation of TAA or peptides to immune cells, on the one hand, and increasing activation or recruitment of specific effector cells at the site of vaccination on the other, using genetically engineered tumor vaccines, is attractive. It has already been applied in patients with disseminated inoperable tumors and will shortly be evaluated for effectiveness in preventing metastases [90].

Another possible way of active immunization may be realized by vaccination with tumor cells transduced with genes for TAA [69]. The rationale behind this strategy is the augmentation of sites on the tumor capable of inducing specific T cell response in vivo. This approach might be possible in tumors expressing shared TAA such as mucins or the MAGE family of antigens [7, 68, 69, 89, 154, 160]. Since several cellular adhesion molecules have been shown to be involved in TCR-mediated T cell activation (see above), augmentation of expression by transfection into tumor cells of ligands for cellular adhesion molecules might further increase tumor-specific T cell responses [119]. Most of the strategies discussed above should preferably be performed in the autologous system, but some may also be applicable in allogeneic tumors that share TAA or restriction elements for TCR-mediated recognition. Altogether, a rapidly increasing knowledge of the TCR and cellular activation, especially of the pathways induced by tumor antigen/peptide recognition, are likely to facilitate the development of efficient new in vitro and in vivo strategies for tumor-specific immunotherapy of cancer.

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