Cytotoxic T Cells

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Cytotoxic T Cells

Mads Hald Andersen[1], David Schrama[2], Per thor Straten[1] and Jürgen C. Becker[2]

The immune system is a complex arrangement of cells and molecules that preserve the integrity of the organism by elimination of all elements judged dangerous. Within the immune system, a humoral and a cellular as well as an innate and an adaptive arm can be differentiated. The key players of adaptive cellular immune responses are T lymphocytes in general and, for the effector function, cytotoxic T lymphocytes (CTLs) in particular. T lymphocytes arise in the bone marrow and migrate to the thymus for maturation. During this process, T cells somatically rearrange gene segments, eventually leading to the expression of a unique antigen-binding molecule, the T-cell receptor (TCR). This receptor allows them to monitor all cells of the body, ready to destroy any cell posing a threat to the organism. Cytotoxicity is exerted directly through the Fas or perforin pathway and/or indirectly by the release of cytokines. Obviously, the activity of such a potent cell is tightly regulated. Indeed, a predominance of stimulatory over inhibitory signals is required for effective immune responses to pathogens, and a predominance of inhibitory over stimulatory signals is required for maintenance of self-tolerance. Still, several situations occur in which an inappropriate CTL response leads to either autoimmune disease or persistence of pathogens.

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The immune system is a remarkably adaptive defense system that has evolved in vertebrates, protecting the host against invading pathogenic microorganisms. This is achieved in a dynamic network of an enormous variety of cells and molecules capable of specifically recognizing and eliminating a large variety of antigens. Once a pathogen has been recognized, the immune system enlists the participation of a variety of cells and molecules to mount an appropriate response to eliminate or neutralize the threat. Subsequent exposure to the same pathogen induces a memory response, characterized by a more rapid and heightened immune reaction.

Immunity includes both nonspecific and specific components. The nonspecific component, innate immunity, represents a set of resistance mechanisms against infection that are not specific to a particular pathogen. In contrast, the specific component, adaptive immunity, displays a high degree of specificity as well as the remarkable property of memory. Because the initiation of an adaptive immune response requires some time, innate immunity provides the first line of defense during the critical period just after the host's exposure to a pathogen. Innate immunity and adaptive immunity do not operate independently of each other but rather work together to elicit an effective immune responses. For example, the action of phagocytes can generate "danger" signals that stimulate and direct the adaptive immune responses, and it can display the phagocytosed antigen in a manner that allows it to be recognized by antigen-specific T cells. Likewise, after encountering appropriately presented antigen, some T cells synthesize and secrete cytokines that may activate macrophages, increasing their ability to kill ingested microbes, a function of the innate immune response.

The adaptive immune system consists of a cellular and a humoral branch. The former is largely mediated by T cells, the latter by B cells. Both cell types arise from the bone marrow. However, unlike B cells, T cells migrate to the thymus for maturation. Still, even when matured T lymphocytes leave the thymus, they are considered naive cells until exposed to antigen in a suitable context. After maturation in the thymus, the T cell expresses a unique cell surface antigen-binding molecule called the T cell receptor (TCR). This antigen-binding molecule consists of two transmembrane molecules, the TCR-α and the TCR-β, that are the result of rearrangement of first the TCR-β and then the TCR-α gene. In contrast to membrane-bound antibodies on B cells, which can recognize antigen alone, the vast majority of TCRs recognize a complex ligand that includes an antigenic peptide bound to a major histocompatibility complex (MHC)-derived molecules (Moss *et al.*, 1992) (Figure 1a). The MHC is a cluster of genes arrayed within a longitudinal stretch of DNA on chromosome 6 in humans and chromosome 17 in mice, the product of which plays a central role in intercellular recognition and discrimination between self and non-self. Two major forms of

[1]*Tumor Immunology Group, Institute of Cancer Biology, Danish Cancer Society, Copenhagen, Denmark; and* [2]*Department of Dermatology, School of Medicine, Julius-Maximilians University, Würzburg, Germany*

Correspondence: Dr. Jürgen C. Becker, Klinik und Poliklinik für Dermatologie, Allergologie und Venerologie, Josef-Schneider-Strasse 2, 97080 Würzburg, Germany. Email: becker_jc@klinik.uni-wuerzburg.de

Abbreviations: APC, antigen-presenting cell; CTL, cytotoxic T lymphocyte; CTLA-4, cytotoxic T lymphocyte-associated antigen 4; DC, dendritic cell; MHC, major histocompatibility complex; Th, T helper

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these polymorphic membrane-bound glycoproteins exist, MHC class I and class II molecules. Class I and class II molecules interact with different co-receptors on the T cells, that is, CD8 and CD4, respectively. Whereas class I molecules are expressed by nearly all nucleated cells, class II molecules are constitutively expressed only by antigen-presenting cells (APCs); however, they can be induced in the majority of cells, in particular by IFN-γ.

In general, mature T lymphocytes express either CD4 or CD8 molecules, hence allowing for identification of $CD4^+$ T helper (Th) cells and $CD8^+$ cytotoxic T lymphocytes (CTLs). Th cells produce cytokines required for triggering of the humoral and the cell-mediated immune response. In this regard, activation of Th cells is carefully regulated, and naive $CD4^+$ cells become activated only upon encountering antigen presented by HLA class II complexes in the context of appropriate co-stimulatory molecules on the surface of professional APCs such as dendritic cells (DCs) (Stockwin *et al.*, 2000) (Figure 1b). Moreover, their differentiation is actively regulated by a small but highly potent sub-population of T cells. These regulatory T cells have recently received much attention for their role in prevention of autoimmunity (see Beissert *et al.*, this issue). The role of the $CD8^+$ T cells is to monitor all the cells of the body, ready to destroy any that is considered to be a threat to the integrity of the host; for example, CTLs kill virally infected cells, preventing them from being the source of more viral pathogen.

Moreover, CTLs are also thought to provide some degree of protection against spontaneous malignant tumors, by virtue of their ability to detect quantitative and qualitative antigenic differences in transformed cells. In this regard, transformation results in an altered protein repertoire inside the cell. $CD8^+$ T cells recognize antigen that is presented by MHC class I-derived molecules that sample peptides from protein degradation inside the cell and present these at the cell surface to CTLs; this enables CTLs to scan for cellular alterations (Castelli *et al.*, 2000).

The aim of this review is to provide an overview of CTLs and their function in physiological and pathological situations. Functionally, an immune response can be divided into two related activities, recognition and response. Consequently, we first describe how T cells recognize their targets and how they become activated. The next section describes T cell-mediated cytotoxicity. This is followed by a description of the markers that help to identify the different types and activation states of T cells. Finally, we try to outline how an understanding of CTL biology may be translated into the clinical situation. CTLs have an impact on a wide variety of clinical situations, such as autoimmunity allergic reactions and infectious and neoplastic diseases. However, the largest body of evidence has been obtained over the years in the field of tumor immunology; thus, we focus the clinical part of the review on this field.

TCR-Mediated Recognition of Target Cells

In 1996, Zinkernagel and Doherty were awarded the Nobel Prize in Medicine for demonstrating that $CD8^+$ T cells could only recognize virally infected target cells if they expressed a particular set of MHC molecules (Zinkernagel, 1997). In humans, all nucleated cells express up to six different class I molecules, which are encoded on chromosome 6 in the MHC locus; the human MHC is referred to as HLA complex and contains three loci (HLA-A, -B, and -C). The MHC molecules are able to present a vast range of peptides. It is estimated that there are up to 250,000 of each HLA class I molecule on the surface of a cell (Parham and Ohta, 1996).

The expression of mature, peptide-loaded class I molecules at the cell surface requires coordination of three essential processes: first, the proteasomal degradation of endogenous proteins to peptides in the cytosol; second, the translocation of peptides across the endoplasmic reticulum membrane; and third, the assembly of the MHC heavy chain with the antigenic peptide and the associated molecule β_2 microglobulin, and the transport of the latter to the cell surface (Figure 2a). In addition to this classical MHC class I pathway in which the antigens presented

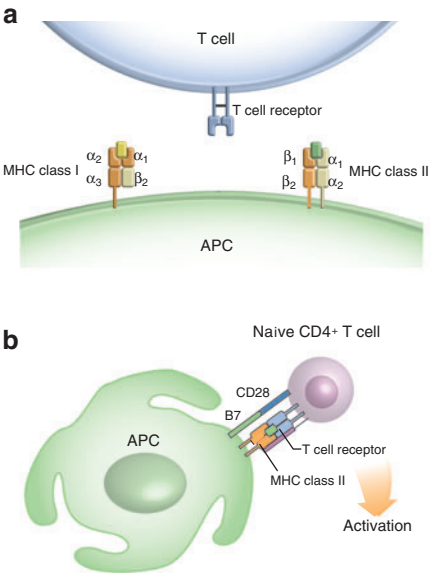

Figure 1. Peptide presentation and T-cell activation. The TCR recognizes antigenic peptides presented in the context of major histocompatibility complex (MHC) (**a**). TCR-mediated recognition of a peptide antigen bound to an MHC-derived molecule represents a central event for cellular immune responses. Cytotoxic T lymphocytes (CTLs) can destroy any cell that expresses the respective peptide/MHC class I complex. As HLA class I complexes are present on most nucleated cells, activation of CTLs is carefully regulated; that is, $CD4^+$ cells produce cytokines required for triggering of CTL response. For $CD4^+$ T helper (Th) cells, the TCR reacts with peptides presented by MHC class II, which have a more restricted expression pattern. Moreover, upon TCR triggering, naive Th cells become activated only in the presence of a co-stimulatory signal mediated, for example, by B7 and CD28 present on the surface of professional antigen-presenting cells (APCs) such as dendritic cells (**b**).

are of intracellular origin, processing of exogenous antigens for presentation by MHC class I molecules is now a widely recognized alternative pathway (Reimann and Kaufmann, 1997). This pathway, which is probably restricted to APCs, is termed the alternative MHC class I pathway, or cross-presentation (Figure 2b). In this pathway, professional APCs such as DCs, which are essential for the induction of CTL responses, internalize exogenous antigens by endocytosis and present fragments of these antigens in the context of MHC molecules to various effector cells of the immune system. It is generally assumed that endocytosed antigens, either before or after processing, may

exit the endosomal pathway into the cytosol, where they can enter the classical MHC class I presentation pathway (Wilson and Villadangos, 2005); direct evidence proving this notion, however, still remains elusive.

TCR-mediated recognition of a peptide antigen bound to an MHC-derived molecule represents a central event for cellular immune responses. The TCR contacts the MHC complex through the TCR variable domains (Garboczi *et al.*, 1996). The affinity between TCR and peptide/MHC is orders of magnitude weaker than that of comparable antibody–antigen interactions. However, because the MHC molecules serve not only as ligands for the TCR, but also as non-antigen-specific ligands for the TCR co-receptors — for example, class I molecules for CD8 and class II molecules for CD4 — even these weak interactions are sufficient to initiate signal transduction in CTLs. Indeed, in CTLs the CD8 molecule has two functions as a receptor, both recognizing the ligand and initiating signal transduction. As is outlined in the following section, CD4 and CD8, however, are not the only co-receptors that are important in the activation of $CD8^+$ CTLs.

A single TCR can recognize a variety of peptide/MHC ligands. Agonist ligands are peptide/MHC complexes that induce complete TCR signal transduction, resulting in widespread changes in gene expression. Antagonist ligands inhibit responses induced by the agonist. In addition, a subset of ligands are inhibitory for multiple cellular responses, such as proliferation and cytokine production, but at the same time induce some alterations in gene expression and functional response in T cells. Indeed, they induce a subset of the responses triggered by agonist ligands and therefore have been named partial agonists. Partial-agonist signaling can lead to cellular responses that are qualitatively different from those induced by agonist signaling. For example, naive T cells proliferate and differentiate in response to agonist, whereas partial-agonist signals lead to survival without significant proliferation or differentiation. In this respect the TCR is unique, as different cellular responses are determined by signaling through a single receptor that interprets subtle differences in ligand structure.

Co-Stimulatory Signals and Cytotoxic T-Lymphocyte Activation

Crucial elements of cellular immune responses are the activation, clonal expansion and differentiation of T cells. The signal transduction events during activation of CTLs and Th cells are generally similar. In addition, activation of either population of T cells is not an independent process but may largely be influenced by the other. Activation of CTLs can be divided into two phases, reflecting different aspects of the response. The first phase activates naive T cells and differentiates them into functional effector cells; in the second phase, these effector cells recognize antigen on specific target cells, which results in the destruction of the target cell (see "Killing of Target Cells" below).

T-cell activation is a complicated process that includes mobilization of Ca^{2+}, new transcription, release of preprocessed and retained surface receptors, internalization of surface receptors, altered susceptibility to apoptosis, and the release of granules (containing perforin and so on).

At different stages of differentiation, T cells may respond with different efficiencies to signals mediated by the TCR and may therefore require different levels of co-stimulatory signals for activation. For example, activation of naive T cells and their subsequent differentiation into effector cells require the primary signal via the TCR and CD4 or CD8 co-receptors and co-stimulatory signals. In contrast, antigen-experienced cells are able to respond to TCR-mediated signals with little, if any, co-stimulation. These differences are at least in part due to the expression of distinct isoforms of CD45, that is, CD45RA and CD45RO (see also " T-Cell Markers" below). These isoforms are generated by alternative splicing of the mRNA transcript, and the resulting proteins exert a phosphatase activity that catalyzes dephosphorylation of the protein tyrosine kinases Lck and Fyn (Figure 3), which activates these kinases and triggers subsequent steps of T-cell activation. Notably, CD45RO, expressed on memory T cells, exhibits

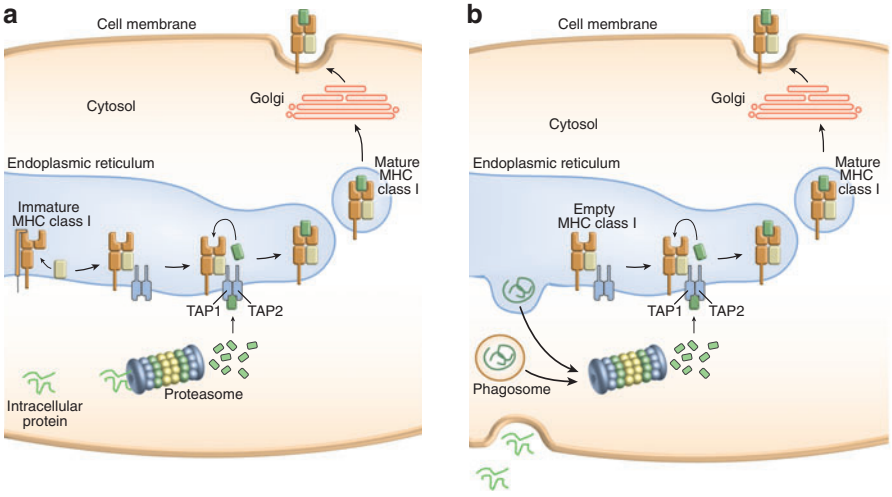

Figure 2. MHC class I antigen processing and antigen presentation pathways. In general, MHC class I-presented peptides are derived from intracellular proteins (**a**). These are degraded by the proteasome and transported through the transporter associated with antigen processing (TAP) in the endoplasmic reticulum. There, newly synthesized MHC class I molecules are stabilized by calnexin until β_2 microglobulin binds to the complex. The partial folded MHC class I complex binds to the TAP complex, and, after binding of peptide, the peptide/MHC complex is transported through the Golgi apparatus to the cell surface. Alternatively, exogenous proteins are phagocytosed, and endocytosed antigens may exit the endosomal pathway into the cytosol, either before or after processing, where they can enter the classical MHC class I presentation pathway (**b**). These proteins are retro-transported out of the endoplasmic reticulum and degraded by the proteasome. The degraded peptides can now enter the normal pathway through the TAP complex.

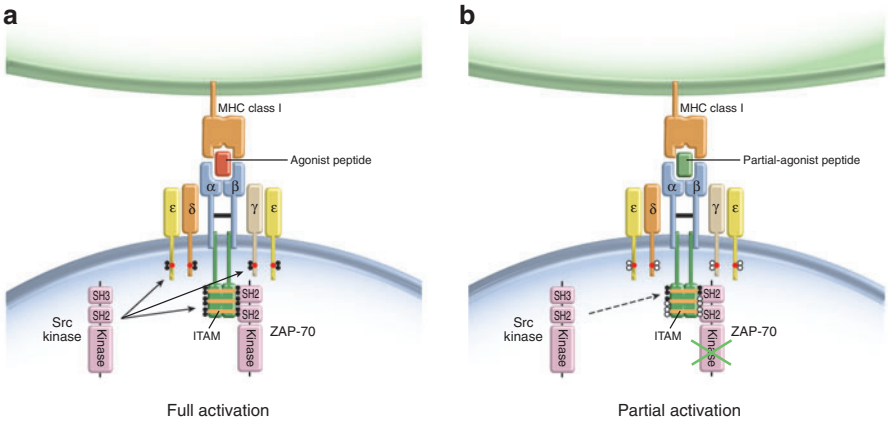

Figure 3. Full and partial T-cell activation. TCR ligation by immunogenic peptides leads to phosphorylation (closed circles) of all immunoreceptor tyrosine-based activation motifs (ITAMs) of the CD3 complex, recruitment, and/or activation of the src kinases and thus full T-cell activation (**a**). Altered peptide ligand engagement causes incomplete phosphorylation of CD3 ITAMs (open circles) and ineffective recruitment of src kinases (**b**). Red circles represent phosphorylation sites. Therefore, some SH2 domain-containing proteins may not be able to bind to the signaling complex.

stronger association with the TCR and its co-receptors than CD45RA, improving the efficiency of the TCR signaling. Additionally, the nature of the epitope also influences the outcome of TCR-mediated signaling. Complete peptide agonists stimulate the cell to perform all of these functions. Partial-agonist peptides are defined as ligands that induce a measurable T-cell response while inducing minimal proliferation or no proliferation. Many models of T-cell activation have been proposed to explain the differential effects of altered peptides. These models include changes in the kinetics of signal transduction, formation of the immunological synapse, induction of a negative signal and recruitment of TCR or co-stimulatory molecules. In addition, the induction of cytolysis without stimulation of T-cell proliferation can also be triggered by peptides with decreased affinity for MHC; this highlights the interaction between peptide and MHC as an important factor in determining the outcome of TCR engagement. Thus, it should be noted that peptide antagonists not only compete for binding to MHC class I molecules but also alter the cascade of processes that result in T-cell activation (Kilgore *et al.*, 2004) (Figure 3). Generation of effector CTLs appears to require at least three sequential signals: (1) TCR ligation; (2) co-stimulatory signals transmitted, for example, by CD28–B7 interactions; and (3) IL-2-mediated signaling. Unactivated naive $CD8^+$ T cells do not express IL-2 or its receptors, which are induced only upon TCR- and CD28-mediated signaling. The amount of IL-2 production induced by these signals, however, may not be sufficient to ensure full activation. In such cases, activated Th cells may provide additional IL-2.

The best-characterized co-stimulatory signaling system required for the activation of naive T cells is based on the interaction of B7 with CD28 and CTL-associated antigen 4 (CTLA-4) (Figure 1b) (Greenwald *et al.*, 2005). Nevertheless, this pathway is rather complex because of the dual specificity of B7-1 (CD80) and B7-2 (CD86) for the stimulatory receptor CD28 and the inhibitory receptor CTLA-4 (CD152). B7–CD28 binding delivers signals important for activation of naive T cells, whereas CTLA-4 inhibits T-cell responses and regulates peripheral T-cell tolerance. CD28 is constitutively expressed on the surface of T cells, whereas CTLA-4 expression is rapidly upregulated after T-cell activation. CTLA-4 has a higher affinity for both B7-1 and B7-2 than does CD28. The expression of other CD28 family members, for example, ICOS, can also be induced on T cells, and they have important roles in regulating previously activated T cells. Thus, CD28 and ICOS synergize to promote the activation of T-cell responses, with CD28 having a predominant role during initial T-cell activation and ICOS regulating memory T cells. Importantly, B7–CD28 interactions have a critical role in the homeostasis of $CD4^+CD25^+$ regulatory T cells, which regulate self-tolerance and T-cell activation (Lohr *et al.*, 2003; Beissert *et al.*, this issue). In addition, recent studies demonstrate that after engagement of CTLA-4, B7 can transmit suppressive signals into DCs by reverse signaling (Greenwald *et al.*, 2005).

Signaling through CD28 molecules reduces the number of TCR–peptide/MHC interactions necessary for T-cell activation; in addition, other membrane-bound or soluble molecules modulate the activation of T cells, either by direct signaling or by increasing the affinity of cell-to-cell interaction. The expression of MHC class I on the cell surface of the APC and the expression of a relevant cytokine by the APC may also facilitate CTL activation. Th cells have also been shown to play an important role in the generation of CTLs. In 1998, it was shown that signaling through the CD40 that is expressed on mature DCs is the prime means of Th cell-dependent preparation of APCs for CTL generation. Schoenberger and colleagues demonstrated that priming of CTL responses against a tumor antigen critically depended on this CD40 signaling (Schoenberger *et al.*, 1998). This CD40-mediated signaling of APCs, however, did not require a cognate T-cell interaction but could be substituted by crosslinking of CD40 with an antibody. Similarly, Matzinger's group demonstrated that DCs treated *in vitro* with an anti-CD40 mAb could be used to prime the Th cell-dependent CTL response to the H-Y antigen in mice deficient in Th cells (Ridge *et al.*, 1998). Further characterization of the effect of CD40 signaling on DCs revealed the downstream events necessary for APC licensing. For example, IL-12, a strong amplifier of CTL responses, is induced by CD40 ligation (O'Sullivan and Thomas, 2003). Similarly, the expression of the co-stimulatory 4-1BB ligand on activated T cells is a consequence of CD40 signaling (Laderach *et al.*, 2003).

In summary, activation of CTLs is a result of binding of TCR to antigen, signaling through a variety of co-stimulatory molecules on the T cell, and signals generated from APCs and Th cells. The efficiency of CTL activation is due in large part to the nature of the TCR stimulation as well as the co-stimulatory signal.

T-Cell Markers

As is outlined above, activation of CTLs results not only in proliferation but also in differentiation of the activated cell. To this end, circulating $CD8^+$ T cells can be divided into four groups, naive, effector, effector/memory, and memory cells, each of which represents a distinct activation/differentiation status of a given T-cell clone. These different states of T-cell activation are associated with distinct functional and phenotypic characteristics (Figure 4). For example, naive T cells circulate only between the peripheral blood and lymphatic tissues, whereas some of the antigen-experienced sub-populations are able to enter other tissues. Furthermore, effector T cells express high levels of perforin, have strong cytolytic activity and produce low levels of cytokines such as IL-2 and IFN-γ, whereas effector/memory cells display medium levels of perforin, allowing only a limited cytotoxic activity, but produce high levels of cytokines (Tomiyama *et al.*, 2002) (Figure 4). In contrast, memory $CD8^+$ T cells fail to kill target cells but can proliferate and produce cytokines in response to antigen stimulation (Kaech *et al.*, 2002).

In addition to functional characterization, there are cell surface markers that have proven helpful in the classification of the different T-cell populations (Figure 4). Thus, a phenotypic classification of human $CD8^+$ T cells using the co-stimulatory receptors CD27 and CD28 as well as CD45RA or CD45RO has been validated as useful for the distinction of naive, memory, and effector $CD8^+$ T cells (Tomiyama *et al.*, 2002). In this regard, naive $CD8^+$ T cells express CD27, CD28, and CD45RA, whereas memory cells lose the expression of CD45RA. Effector $CD8^+$ T cells are $CD27^-CD28^-$ $CD45RA^+$, and effector/memory cells have a $CD27^-CD28^-CD45RA^-$ phenotype (Sobao *et al.*, 2001). This notion has been substantiated by *in situ* studies scrutinizing the phenotype of individual T-cell clones present in either the draining lymph node of cutaneous melanoma or the tumor itself (Becker *et al.*, 2000). In addition, it was recently suggested that $CD27^+CD28^-CD45RA^-$ $CD8^+$ T cells are effector/memory cells as well, as they have cytotoxic activity and can effectively produce cytokines (Tomiyama *et al.*, 2002).

The chemokine receptor CCR7 is a particularly useful marker for discriminating naive and memory $CD8^+$ T cells from effector/memory and effector $CD8^+$ T cells (Champagne *et al.*, 2001). CCR7 functions as a homing receptor to lymphoid tissue and is expressed on naive $CD8^+$ T cells and a subset of memory $CD8^+$ T cells. Thus, naive ($CCR7^+CD45RA^+$), central/memory ($CCR7^+CD45RA^-$) and effector/memory ($CCR7^-CD45RA^{+/-}$) cells can be distinguished (Tomiyama *et al.*, 2004).

It has been suggested that $CD8^+$ cells switch from naive to memory status in response to antigen exposure and then gradually transform into an effector type (reviewed by van Baarle *et al.*, 2002) (Figure 4). The effector phenotype is $CD45RA^{high}CD27^-CD28^-CCR7^-$ and expresses high amounts of perforin, IFN-γ and granzyme. Furthermore, it has been shown that the majority of circulating immunization-induced $CD8^+$ T cells display an intermediate effector/memory $CD45RA^{high}CD27^-$ phenotype. These cells are capable of expressing IFN-γ but demonstrate little or no expression of perforin. Notably, CD27 and perforin expression can be re-induced by *in vitro* sensitization associated with increased cytoplasmic size and significantly decreased frequency of $CD45RA^{high}$ cells (Monsurro *et al.*, 2002). These tumor antigen-specific $CD8^+$ T cells possess neither a distinct memory nor a distinct effector phenotype (Monsurro *et al.*, 2002), as full effector functions can be regained only when appropriate conditions are provided *in vitro*, for example as previously described for antigen recall in the presence of IL-2 (Kammula *et al.*, 1999). Thus, it is possible that the transient status of quiescence of immunization-induced T cells can be overturned by the administration of cytokines such as IL-2. In fact, both systemic administration and tumor-targeted enrichment of IL-2 significantly improved therapeutic vaccinations with tumor peptide-pulsed DCs (Eggert *et al.*, 2002; Schrama *et al.*, 2004). The improvement of the therapeutic effect can be ascribed to the ability of IL-2 to boost preexisting immune response, probably by reversing the quiescent state of tumor-specific T cells (thor Straten *et al.*, 1998).

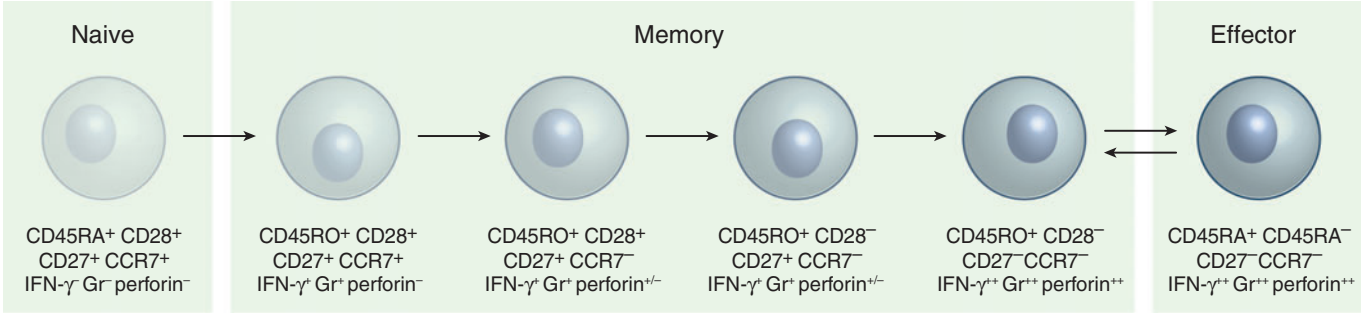

Figure 4. T-cell differentiation. CD8 T cells can be distinguished by the expression of different molecules such as CD45RA, CD45RO, CD28, CD27, or CCR7, or mediators of cytotoxicity such as IFN-γ, granzyme (Gr) or perforin.

Killing of Target Cells

CTLs may kill target cells by one of at least three distinct pathways. Two involve direct cell–cell contacts between effector and target cells. The third is mediated by cytokines, such as IFN-γ and tumor necrosis factor-α, which are produced and secreted as long as TCR stimulation continues (Figure 5a). These cytokines affect the opposed target cell or cells distal to the effector T cell. Tumor necrosis factor-α engages its receptor on the target cell and triggers the caspase cascade, leading to target-cell apoptosis. IFN-γ, however, induces transcriptional activation of the MHC class I antigen presentation pathway and Fas in target cells, leading to enhanced presentation of endogenous peptides by MHC class I, and increases Fas-mediated target-cell lysis. Cytolytic activity requiring direct cell–cell contact, which results in apoptosis of target cells, can be mediated by two different mechanisms. In one case, the Fas ligand, which is expressed on the surface of CTLs, binds to the Fas receptor (Fas, CD95) on the target cell (Figure 5b). This binding triggers apoptosis through the classical caspase cascade (Nagata, 1996). In the other case, the CTL releases perforin and granzymes into the intercellular space (Figure 5c). These proteins are highly cytotoxic, and the CTLs have devised an elaborate mechanism to protect themselves and neighboring cells from being killed accidentally while still ensuring that the cell can show a rapid and efficient cytotoxic response upon triggering the TCR. Firstly, the majority of the cytotoxic proteins are pre-synthesized, and so ready to be used in killing upon encountering a target cell. The regulated secretory organelles in which the lytic proteins are stored mobilize themselves to the cell surface and expose their content only upon contact with a target. CTLs use their lysosomes as the regulated secretory organelles, which are therefore often referred to as secretory lysosomes (Blott and Griffiths, 2002). Secondly, the secretory lysosomes do not exocytose their content randomly over the cell surface but are mobilized to a defined point on the plasma membrane that is immediately opposite the target cell, termed the secretory domain. Thirdly, the secretory lysosomes release their content not into the general extracellular milieu, from where they could diffuse and kill other innocent neighboring bystander cells, but into a defined space, or "cleft," that forms between the otherwise tightly opposed CTL and target-cell membranes. This *modus operandi* therefore both concentrates the cytotoxic proteins for maximum impact and confines them to the environment of the target cell. The uptake of the granular material by the target cell causes cell death in a caspase-dependent and -independent manner (Trapani and Smyth, 2002).

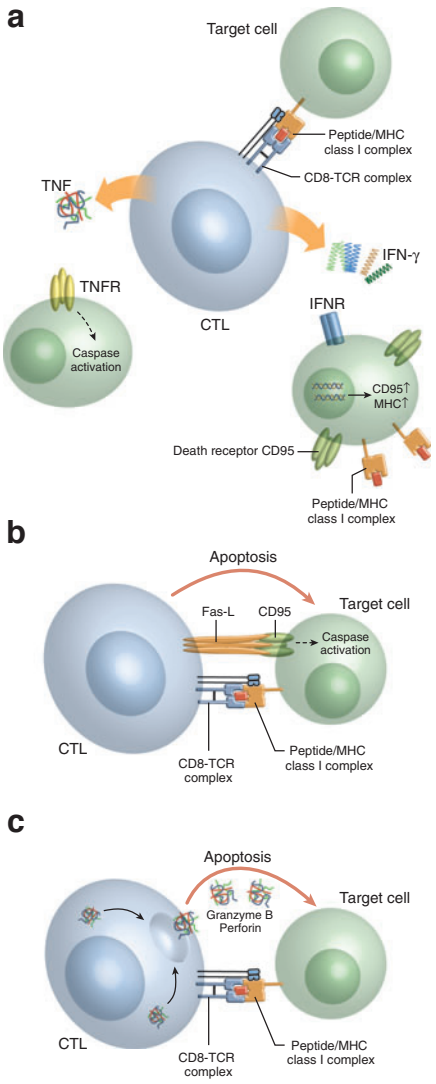

Figure 5. CTL-mediated cytotoxicity. (**a**) Indirect killing of target cells by release of tumor necrosis factor-α and IFN-γ. (**b**) Induction of apoptosis in target cells via death receptor triggering. (**c**) Direct killing by release of granzyme B and perforin into the intercellular space between CTL and target cell. TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor; IFNR, IFN receptor; Fas-L, Fas ligand.

Cytotoxic T Lymphocytes and Disease: Type IV Hypersensitivity

Allergic contact dermatitis is a delayed-type hypersensitivity reaction mediated by hapten-specific T cells (De Panfilis, 1998). During the sensitization phases, both $CD4^+$ and $CD8^+$ T-cell precursors are activated in the draining lymph nodes by presentation of haptenated peptides. Re-challenge with the hapten induces the recruitment of T cells at the site of challenge, that is, the skin; this induces inflammatory signals and apoptosis of epidermal cells, leading to the development of a skin inflammatory infiltrate and to clinical symptoms (Girolomoni, 2004). The respective roles of $CD4^+$ and $CD8^+$ T cells in the development of the inflammatory reaction are still the subject of controversial discussions (Cavani, 2005; Saint-Mezard *et al.*, 2004). Experimental evidence, however, suggests that in contact hypersensitivity responses to strong haptens, $CD8^+$ T cells are the key effector cells, whereas $CD4^+$ T cells are endowed with downregulatory functions. Ongoing studies will have to confirm that the pathophysiology of human allergic contact dermatitis is indeed comparable to the murine contact hypersensitivity.

Cytotoxic T Lymphocytes and Disease: Autoimmunity

Organ-specific autoimmune diseases are caused by an immune response directed to a target antigen unique to a single organ; thus, manifestations are largely limited to that organ. Such autoimmune diseases may involve direct cellular damage mediated by antibodies and/or CTLs. For example, in Hashimoto's thyroiditis, both antibodies and T cells are involved (Brazillet *et al.*, 1999; Farzati *et al.*, 2005). In insulin-dependent diabetes mellitus and multiple sclerosis, the etiological function of CTLs is well established (Neumann *et al.*, 2002). Furthermore, recent reports suggest a function of CTLs in the pathogenesis of skin diseases such as systemic sclerosis and paraneoplastic

pemphigus (Hoffman *et al.*, 2003; Tamby *et al.*, 2003). However, the strongest and most direct evidence for a key role of CTLs in the pathogenesis of autoimmune disease exists for vitiligo. Vitiligo is a common hypopigmentary disorder that affects approximately 2% of the world population (Hartmann *et al.*, 2004). The loss of skin pigmentation is caused by the selective destruction of melanocytes. The initiation and the ultimate pathway of melanocytic destruction in vitiligo are, however, not fully understood. Observations of vitiligo accompanying metastatic melanoma (melanoma-associated hypopigmentation) support a role of autoreactive CTLs recognizing shared melanocyte differentiation antigens (Becker *et al.*, 1999; Pedersen *et al.*, 2002; Steitz *et al.*, 2004).

Cytotoxic T Lymphocytes and Disease: Tumors

The largest body of evidence that CTLs are involved in the clinical course of disease exists for neoplastic disease. Most of the work focuses on the function of CTLs in the control (or lack of control) of tumors. In 1982, Van Pel and Boon demonstrated the generation of a protective immune response against an otherwise non-immunogenic murine tumor. This was the first experimental evidence that lack of immunogenicity of a tumor is due to the tumor's inability to activate the immune system rather than to the absence of tumor antigens (Van Pel and Boon, 1982). Since then it has become clear that $CD8^+$ CTLs alone or sometimes in combination with $CD4^+$ Th cells constitute the effector arm of the adaptive immune response to cancer (Castelli *et al.*, 2000). Thus, it is not surprising that much effort is spent on identification and characterization of tumor antigens for use in active immune therapy. To this end, the number of known T-cell epitopes derived from tumor-associated antigens exceeds 200 and is still increasing (Novellino *et al.*, 2004). The proteins from which these peptide antigens are derived can be divided into different groups. It should be noted, however, that the distinctions between some of the groups are somewhat arbitrary.

Group I. A number of patient-specific antigens have arisen as a result of somatic mutations in normal gene products. These antigens can be expressed by single tumors only (unique) or shared among tumors of the same entity (reviewed by Renkvist *et al.*, 2001).

Group II. Group II consists of tumor-specific antigens that nevertheless are shared among cancer patients. These antigens can be further divided into two subgroups. One subgroup consists of viral antigens present in several cancers, such as Epstein-Barr virus in lymphoma and human papilloma virus in cervical cancer (Boon and van der Bruggen, 1996). The other subgroup consists of antigens that have arisen as a result of mutations related to the oncogenic process and therefore are present in a large proportion of patients. These antigens are mutated proteins, such as p53 (Ciernik *et al.*, 1996), ras (Bergmann-Leitner *et al.*, 1998), and bcr/abl (Yotnda *et al.*, 1998). For this subgroup we recently described spontaneous CTL responses to the oncogenic ^{V600E}BRaf mutation in melanoma patients, resulting in a selective outgrowth of melanoma cells lacking this mutation (Andersen *et al.*, 2004).

Group III. The largest number of antigens identified so far are shared tumor antigens that correspond to normal tissue-specific gene products, also called differentiation antigens. Such antigens have been isolated from melanoma and are also expressed by normal melanocytes. They include MART-1/Melan A, gp100 and tyrosinase (Castelli *et al.*, 2000; Van den Eynde and Boon, 1997).

Group IV. This group of antigens consists of normal proteins that are predominantly expressed by tumors whereas they are not present in normal tissue, with the exception of testis. Prototypes of this group are the MAGE, GAGE, and BAGE families (Boon and van der Bruggen, 1996; Boel *et al.*, 1995; Van den Eynde and Boon, 1997). A number of such antigens are overexpressed only in particular cancers, for example, the HER-2/neu oncogene, which is frequently overexpressed in adenocarcinomas of breast, ovary, and colorectum. Recently, a number of antigens were identified that are not restricted to a subgroup of tumors but are expressed in most if not all of the common human malignancies. For example, Vonderheide *et al.* demonstrated human CTL responses against telomerase, a protein necessary to avoid replicative senescence of cancer cells (Vonderheide *et al.*, 1999). Likewise, T-cell responses against the antiapoptotic protein survivin have been described (Schmitz *et al.*, 2000; Andersen *et al.*, 2001a; Andersen *et al.*, 2001b).

Based on the observation that tumors are immunogenic, but that the induced immune responses are not capable of controlling clinically evident lesions, a large research effort has been launched to boost antitumor immune responses. In this regard, the T-cell growth factor IL-2 is being used to treat patients with renal-cell carcinoma and melanoma. Although response rates have been minute, some patients have experienced durable responses (Atkins *et al.*, 1999). This has set the stage for additional efforts in immune therapy of cancer, including the use of defined peptides in the specific targeting of cancer cells, for example, therapeutic vaccination. Indeed, several immunotherapeutic trials have been initiated for treatment of various malignancies, for instance, melanoma, cancers of the breast, prostate and kidney, and hematological neoplasms. By various strategies it has now been established that it is feasible and safe to induce anticancer CTLs in patients by means of antigen-specific vaccination (Wang *et al.*, 2001). Notably, although the antigens used for specific vaccination often belong to the class of differentiation antigens, which are also expressed by all melanocytes, widespread vitiligo is a rare event in vaccinated melanoma patients. In contrast, melanoma-associated hypopigmentation is rather frequently observed. A possible mechanism preventing generalized vitiligo relies on MHC-specific killer-inhibitory receptors. In this regard, we previously demonstrated the expression of activating and inhibitory receptors of CD94/NKG2 on T cells within the tumor, whereas T cells in the vitiligo-like leukoderma expressed exclusively the inhibitory isoforms

(Pedersen *et al.*, 2002). Nevertheless, the potential targets of tumor-specific T cells have to possess specific features in order to be destroyed by the T cells. In accordance, in mice, DCs consistently trigger autoimmune responses, which, however, lead to clinical autoimmunity only in susceptible animals (Bondanza *et al.*, 2003).

In general, two different vaccination strategies are being pursued: the use of vaccines that utilize whole tumor cells, and the use of vaccines that target defined antigens (Melief *et al.*, 2000). Although the main components of cell-based vaccines are irrelevant proteins, these vaccines hold the advantage that all relevant tumor antigens are covered. However, the complexity of the antigenic composition of tumor cells implies that immunological monitoring of these therapeutic interventions is troublesome. In contrast, vaccines comprising defined antigens allow systematic biological monitoring, thus enabling immediate improvement of vaccine design based on the findings. However, the clinical effect of these defined vaccines has so far been marginal at best. Likewise, the appropriate delivery of tumor antigens in anticancer vaccines is currently under intense investigation. This includes DC-based vaccines or molecularly defined adjuvants; inflammatory cytokines; agents that modulate APC and T-cell function; and DNA-based versus virus-based vaccine vectors (Melief *et al.*, 2000). Nevertheless, many questions still have to be answered before immunotherapy may represent a curative means of treatment of metastatic cancers; hence, it is mandatory to scrutinize vaccine-induced immune responses carefully, to set the stage for development of more efficient next-generation anticancer vaccines.

Cytotoxic T Lymphocytes and Disease: Monitoring Immune Responses

For any treatment of cancer, the desirable end point is tumor regression. However, considering that most patients enrolled in immune therapeutic trials are at a progressed stage of disease, objective clinical end points are not really expected — and rarely seen. Consequently, immunological monitoring over the course of treatment is a crucial step to improve our understanding of antitumor immune responses. The methodological advances over the past few years, including the use of enzyme-linked immunospot (ELISPOT) (Herr *et al.*, 1996; Keilholz *et al.*, 2002), fluorescently labeled multimeric peptide/MHC reagents in multicolor flow cytometry (Betts *et al.*, 2003; Altman *et al.*, 1996), and TCR clonotype mapping (Pannetier *et al.*, 1995; thor Straten *et al.*, 2001; Schrama *et al.*, 2002a), have made it possible to conduct detailed studies to characterize the impact of immune modulation. In this respect, lack of correspondence between immunological and clinical responses represents a critical concern (Schrama *et al.*, 2001). Indeed, tumor-specific CTLs may be present in the circulation in cancer patients despite tumor progression, and vice versa (Parmiani *et al.*, 2002).

Following the characterization of peptide antigens recognized by autologous T cells on the surface of cancer cells, numerous small therapeutic vaccination trials against cancer have been performed, aiming at the induction of clinically relevant antitumor CTL responses. Although some reports have demonstrated impressive clinical responses (Rosenberg *et al.*, 1998), in most cases vaccination has not yet convincingly demonstrated any impact on the course of the disease (Parmiani *et al.*, 2003). Hence, the increased insight into events induced by immune modulation relies on biological monitoring. To this end, an improved understanding of the cells and molecules that govern the success or failure of any given therapy may lead the way to significant improvements of current therapeutic strategies. Consequently, characterization and tracking of the TCRs utilized by tumor-specific T cells may reveal important information as to the biology of antitumor T-cell responses (Andersen *et al.*, 2001b; Schrama *et al.*, 2002b; Schrama *et al.*, 2003). The characterization of systemic tumor antigen-specific T-cell responses alone does not necessarily reflect their localization and function within the target tissue, the tumor site. Monitoring of antitumor immune responses is largely restricted to circulating lymphocytes, because this is the most convenient tissue to work with — and in the majority of cases the only tissue accessible. It is obvious that important information may be obtained from combined analyses of peripheral blood and tumor tissue, in particular because we and others have presented data suggesting a functional dissociation between local and systemic immune responses (Lee *et al.*, 1998; thor Straten *et al.*, 1999). In this respect, it may seem optimal to analyze the target tissue if possible. However, biopsies are not easily accessible, and frequent and/or serial sampling of biopsies is associated with severe discomfort to the patients, in particular when sampling goes beyond skin metastases. In addition, it should be kept in mind that differences between systemic and localized immune responses most likely coexist with dissimilarities between different localized lesions in the same patient (thor Straten *et al.*, 1999). This notion is exemplified by the common finding that some lesions regress whereas others progress in melanoma patients receiving immune therapy. Consequently, combinations of the outlined methods, applied to analyze both blood and tissue for characterization of antitumor CTL responses, may provide the necessary information. Thus, the use of recombinant HLA molecules for isolation and subsequent tracking of peptide-specific clonotypic T cells represents a strong tool to delineate the correlation between the *in situ* and the *in sanguis* situation.

CONFLICT OF INTEREST

The author states no conflict of interest.

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