REVIEW

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Antigen-nonspecific activation of CD8$^+$ T lymphocytes by cytokines: relevance to immunity, autoimmunity, and cancer

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Abstract

Development of T lymphocytes and their survival in the periphery are dependent on signals emanating from cytokine receptors as well as the T cell antigen receptor (TCR). These two signaling pathways play distinct and complementary roles at various stages of T cell development, maturation, survival, activation and differentiation. During immune response to foreign antigens initiated by TCR signaling, cytokines play a key role in the expansion of activated T cells. Even though the initial activation of T cells occurs via the TCR, this requirement can be overcome under certain circumstances. During lymphopenia, cytokines trigger memory CD8$^+$ T cells to undergo antigen non-specific homeostatic expansion, whereas naïve CD8$^+$ T cells require both cytokines and TCR signaling. Recent reports show certain combinations of cytokines can induce proliferation and effector functions of naïve CD8$^+$ T cells without concomitant stimulation via the TCR. While such antigen non-specific stimulation of naïve T cells might significantly boost the adaptive immune response, it could also have an undesirable effect of triggering potentially autoreactive cells. Understanding the mechanisms and the regulation of cytokine-driven stimulation of naïve CD8$^+$ T cells may lead to novel strategies of intervention for autoimmune diseases. On the other hand, *in vitro* expansion of naïve CD8$^+$ T cells by certain combinations of cytokines could be used to generate tumor-specific cells with ideal properties for cellular immunotherapy of cancer.

Key words: CD8$^+$ T lymphocytes, cytokines, immune response, autoimmunity, cancer.

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INTRODUCTION

Activation of T lymphocytes by antigen via the T cell antigen receptor (TCR) and concomitant delivery of co-stimulatory signals are the central requirements to mount an effective immune response against pathogens while discouraging the activation of autoreactive cells. This two-signal requirement can be overcome under certain circumstances like lymphopenia, where cytokines such as interleukin (IL)-15 and IL-7 induce proliferation of T cells [59]. Using transgenic and knockout mouse models, it has been demonstrated that cytokine-driven antigen non-specific homeostatic proliferation does not require TCR signaling in memory CD8$^+$ T cells, whereas interaction between the TCR and the MHC:self-peptide complex is crucial for naïve CD8$^+$ T cells. Recent findings show that naïve CD8$^+$ T cells of human and mouse origin can be stimulated by cytokines without requirement for a nominal antigen [2, 36, 42, 171] (Fig. 1). Such cytokine-activated cells show increased responsiveness to subsequent stimulation via the TCR [1, 35]. Implications of these findings to immune response to microbial antigens, activation of autoreactive T cells and expansion of tumor-specific CD8$^+$ T cells for adoptive cell therapy are discussed.

CYTOKINE REGULATION OF CD8$^+$ T CELL ACTIVATION AND DIFFERENTIATION

CD8$^+$ cytotoxic T lymphocytes (CTLs) not only confer protection against viruses and intracellular bacteria but also contribute to the pathogenesis of autoimmune diseases [48,163]. Activation of naïve CD8$^+$ T cells by antigen occurs in secondary lymphoid organs, and requires activated dendritic cells (DCs), which display

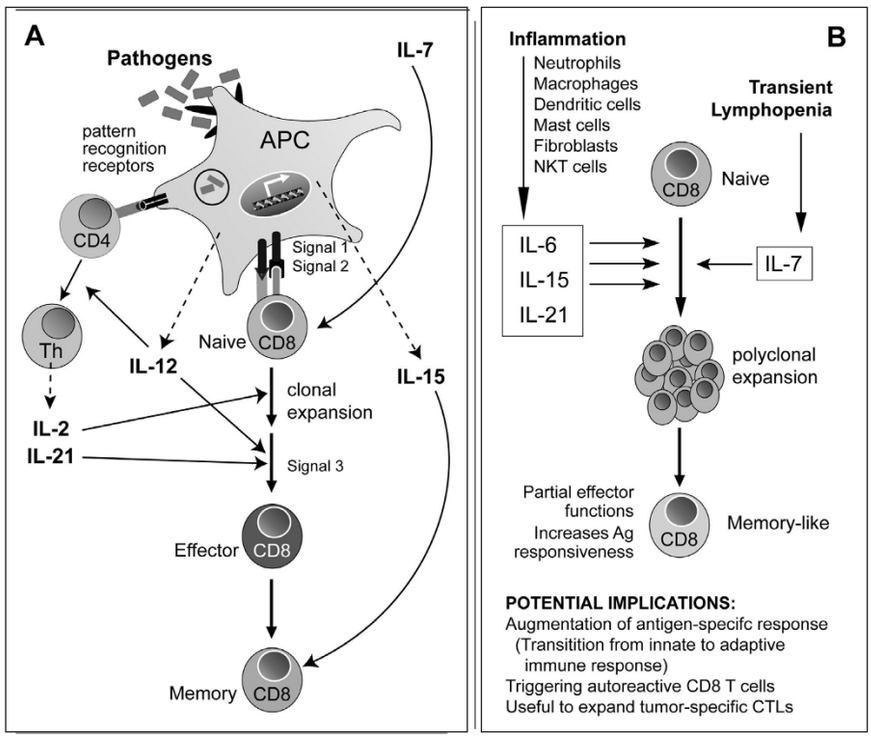

Fig. 1. (**A**) Antigen-specific TCR-dependent activation of naïve CD8+ T cells. Activation of CD8+ T cells in the classical way occurs following stimulation via the TCR by complexes of MHC:antigenic peptides on professional antigen-presenting cells (APC). Activated cells undergo clonal expansion under the influence of IL-2, which is secreted by activated CD4 and CD8 T cells. Signal 3, provided by certain cytokines, is necessary for an optimal CD8+ T cell response to occur. Most of the expanded cells become effector cells and die upon elimination of the antigen. IL-7, secreted by bone marrow stromal cells, promotes differentiation of some of the activated cells into memory cells, which are maintained for a long time by IL-15 expressed by many cell types upon exposure to microbial products. (**B**) Antigen-nonspecific TCR-independent activation of naïve CD8+ T cells. Under inflammatory conditions, IL-15, IL-21 and IL-6 are produced by cells of the innate immune system, while the availability of IL-7 might increase due to downregulation of IL-7R by many other survival cytokines [99]. Certain combinations of these cytokines, for example IL-7 or IL-15 in the presence of IL-21 or IL-6 can stimulate naïve CD8 T cells in the absence of antigen or costimulatory signals. We propose that while antigen-nonspecific activation of T cells recruited to the inflammatory sites might potentiate adaptive immune responses, it would also increase the risk of triggering potentially autoreactive CD8 T cells.

increased co-stimulatory capacity [5]. DCs also 'cross-present' antigens acquired from dying cells to CD8+ T cells [25]. Efficient activation of CD8+ T cells requires help from activated CD4+ T cells, which stimulate maturation and antigen presenting functions of DCs via CD40L [120]. The helper function of CD4+ cells is also critical for the maintenance of CD8+ memory T cells [142].

Naïve CD8+ T cells require only a brief encounter with the antigenic peptide to become activated and to undergo cell division [158]. IL-2, secreted by activated CD4+ and CD8+ T cells, provides paracrine and autocrine stimulation to augment proliferation of antigen-stimulated CD8+ T cells [20]. During this expansion phase, CD8+ T cells undergo programmed differentiation to become effector T cells, which express effector molecules such as perforin, FasL, interferon (IFN)-γ and tumor necrosis factor (TNF)-α [48]. Effector CD8+ T cells also express adhesion molecules, re-enter circulation and disseminate throughout the body to clear the pathogen. At the end of the immune response, activated T cells die through passive and active cell death pathways. Lack of stimulation resulting from elimination of antigen and exhaustion of cytokine resources such as IL-2 and IL-7 lead to a programmed contraction process that eliminates >90% of the expanded CD8+ T cells [3, 83].

During the course of an immune response, approximately 5% of the activated CD8+ T cells differentiate into memory cells to provide long-term immunity [95]. Memory CD8+ T cells may become "effector" (T_{EM}) or "central" (T_{CM}) memory cells under the influence of IL-2 and IL-15, respectively, both in humans and in mice [81, 130]. T_{CM} and T_{EM} subsets differ in their proliferative potential, tissue distribution, migratory patterns, and surface expression of CD62L, chemokine receptor CCR7 and leukotriene B4 receptor receptor BLT1 [81, 130, 165]. Upon re-stimulation, memory CD8+ T cells divide after a short lag time with increased rate of cell division and decreased rate of cell loss, and efficiently upregulate multiple effector functions compared to naïve cells [15, 159].

In the absence of antigen, maintenance of CD8+ memory T cells may depend on bystander proliferation

stimulated by cytokines that are generated during heterologous infections [154]. Other studies have shown that during heterologous virus infections, limited bystander activation of memory CD8+ T cells can occur but it does not increase their pool size [29, 95, 136] and cross-reactive epitopes are critical to maintain the longevity of antigen-specific CD8+ memory T cells [9, 135, 140]. Nonetheless, these studies do not preclude the role of cytokines in maintaining the memory CD8+ T cell pool. The erosion of antigen-specific memory during heterologous infections lacking cross-reactive epitopes [135] could also be explained by the limited space available within the memory cell compartment and competition for cytokine resources.

CYTOKINE REGULATION OF CD8+ T LYMPHOCYTE HOMEOSTASIS

In young adult mice, mature naïve T cells are continuously exported from the thymus into the circulation. In the periphery, T cells can undergo massive proliferation upon encounter with foreign antigens. Despite such continuous influx and frequent but transient expansion, the total number of peripheral T cells remains constant in proportion to the body mass [34, 83, 151]. In normal mice, the ratio between CD4+ and CD8+ T cells in peripheral lymphoid organs is maintained within certain limits, for example, around 2:1 in the spleen and lymph nodes (LNs) of C57Bl/6 mice [98, 122]. However, CD4+ and CD8+ T cells can compensate for each other's loss to maintain a stable number of T cells [34]. Immune response to foreign antigens generates memory T cells in both CD4+ and CD8+ T cell compartments. The naïve and memory T cells occupy different homeostatic niches, the sizes of which remain stable despite continuous thymic output of naïve cells and intermittent addition of memory T cells [150]. Within the naïve and memory compartments, accommodation of new cells occurs by random displacement of the resident cells by mechanisms that are not yet well understood.

T cell homeostasis is regulated by cell survival, proliferation, and death. The factors and the underlying mechanisms regulating these processes are largely similar for CD4+ and CD8+ T cells [83]. Adoptively transferred naïve and memory T cells undergo slow "basal homeostatic proliferation" in normal mice, whereas in T cell deficient/depleted mice they undergo rapid "acute homeostatic proliferation" to swiftly restore the T cell pool [122]. CD8+ T cells undergo homeostatic proliferation faster than CD4+ cells [59, 83]. Naïve CD8+ T cells that undergo acute homeostatic proliferation upregulate the cell surface expression of CD44, CD122 and Ly6C, but not the activation markers CD25 or CD69, thus resembling memory CD8+ T cells [14, 45, 59, 94].

Naïve and memory CD8+ T cells are quite distinct in their requirement for TCR signaling and cytokines to undergo homeostatic expansion. Mice lacking MHC molecules, cytokines or cytokine receptors have clearly established that survival and homeostatic proliferation of naïve CD8+ T cells is dependent on MHC molecules and cytokines, but do not require costimulatory signals [59, 96, 149]. In contrast, survival and renewal of memory cells occur independently of MHC molecules but require cytokines. Two members of the IL-2 family of cytokines, IL-7 and IL-15, play indispensable roles in CD8+ T cell homeostasis. IL-7 is secreted by bone marrow-derived stromal cells, whereas IL-15 is produced predominantly by antigen presenting cells [53, 79]. *IL-7*$^{-/-}$ and *IL-7R*$\alpha^{-/-}$ mice exhibit impaired T cell development and 10 to 100-fold reduction in the number of peripheral T cells [110, 161]. IL-7 is critical for the survival of naïve and memory CD8+ T cells [116, 132, 147]. However, long-term survival of CD8+ memory T cells is critically dependent on IL-15 [6, 77]. Mice lacking IL-15 or IL-15Rα present less severe lymphopenia but display pronounced lack of CD8+ memory T cells [65, 77]. Transgenic expression of IL-7 or IL-15 results in increased number of CD8+CD44^{hi} memory T cells [31, 66]. Using mice lacking IL-7, IL-15 or both, it has been shown that IL-7 mediates homeostatic expansion of memory CD8+ T cells only in lymphopenic hosts, presumably due to lack of competition from CD4+ T cells, whereas IL-15 is essential for proliferative renewal of memory CD8+ T cells in mice with an intact T cell compartment [6, 46, 66, 148]. Recent studies in mice have shown that increased availability of IL-2 or IL-15 caused by specific deletion of the IL-2Rβ (CD122) or the common γ_c chain (CD132), or by extending the half-life of IL-2 by anti-IL-2 mAb can augment antigen non-specific activation of naïve CD8+ T cells *in vitro* and under conditions of lymphopenia *in vivo* [16, 63, 114]. As IL-7 and IL-15 upregulate the expression of the anti-apoptotic proteins Bcl-2 and Mcl-1 [84, 103, 116, 132, 168], accumulation of CD8+ T cells that occurs under conditions of cytokine abundance could result not only from proliferation but also from increased survival.

Triggering a cell death program is essential to restore the normal T cell number following an immune response [157]. Activated T cells are turned off by two distinct mechanisms that involve cytokine receptor signaling [50, 157]. Death receptors Fas and TNFR stimulate activation-induced cell death (AICD), a caspase-dependent process that does not involve Bcl-2. Contrary to its requirement for clonal expansion, IL-2 also promotes Fas-mediated AICD in CD4+ and CD8+ T cells [21, 75]. Accordingly, mice lacking IL-2 or IL-2Rα accumulate activated T cells and develop autoimmunity [129, 143]. Intriguingly, administration of IL-2 during the expansion of T cells in response to lymphocytic choriomeningitis virus infection partially blocked the increase in CD4+ T cells but not CD8+ T cells, and did not compromise the overall immune response, whereas IL-2 injected during the contraction phase sustained the survival of virus-specific CD8+ T cells and enhanced the antiviral response [7]. One possible explanation for the contradictory effect of IL-2 on CD8+ T cell response *in vivo* compared to the *in vitro* observations could be

attributed to IL-15 induced by viral infections because IL-15 has been shown to prevent IL-2-dependent AICD [82]. In contrast to AICD, passive death of activated T cells or "activated T cell autonomous death" (ACAD) is mediated by a decrease in Bcl-2 expression [50, 92]. At the peak of an immune response, cellular Bcl-2 level decreases due to limiting amounts of IL-2. Other γ_c cytokines such as IL-4, IL-7 and IL-15 can improve survival of activated T cells, presumably via upregulating the pro-survival proteins Bcl-2 and Mcl-1 [85, 103, 160].

ANTIGEN NON-SPECIFIC PROLIFERATION OF CD8+ T CELLS MEDIATED BY CYTOKINES

The observation that the slow, steady-state proliferation of memory CD8+ T cells was sharply increased by administration of lipopolysaccharide or poly I:C [153, 155] led to the identification of IL-15 as the principal mediator of memory CD8+ T cell proliferation [172]. Increased sensitivity of memory CD8+ T cells to IL-15 results at least partly from elevated expression of IL--2Rβ chain (CD122), which is a component of the IL--15R complex [8]. Since IL-15 is induced in marophages and DCs by a variety of danger signals, it is conceivable that rapid, bystander stimulation of memory cells would be advantageous to the host if the invading pathogen has been previously encountered. Hence, preferential expansion of memory CD8+ T cells in lymphopenic hosts could be conceived as an attempt to rapidly restore the immune functions directed against pathogens that are likely to be encountered again.

Imposition of the requirement for self MHC molecule and a "basal" level of TCR signaling for homeostatic expansion of naïve CD8+ T cells could be a natural design to discourage expansion of potentially autoreactive cells as well as to allow sufficient time for thymic export to repopulate the periphery with a diverse repertoire of T cells. Therefore, it is reasonable to assume that in naïve CD8+ T cells cytokine signals can only supplement the TCR-dependent stimulation. However, emerging studies suggest that naïve CD8+ T cells are not very stringently dependent on TCR signals to undergo proliferation. Antigen non-specific proliferation of both memory and naïve T cells can be induced in mice with a full T cell compartment by CpG-deoxyoligonucleotides, possibly via induction of T cell-stimulatory cytokines [23]. Cytokine-stimulated antigen non-specific proliferation of naïve human CD4+ T cells was originally described by Geginat et al. [43]. Subsequently, naïve human CD8+ T cells, defined by the $CD45RA^+CCR7^-$ and $CD45RA^+CD27^+$ phenotypes, have been shown to proliferate in response to IL-15 either alone or in combination with IL-7 [2, 42]. Naïve $CD44^{lo}CD122^{lo}$ murine CD8+ T cells do not respond to IL-15 alone, but can be induced to proliferate by IL-15 in the presence of a new member of the γ_c cytokine family, IL-21 [36, 171]. In human naive CD8+ T cells, IL-21 augments the response to IL-15 and sustains the expression of CD28 [1]. IL-21 is produced by activated CD4+ T cells, Th17 cells and NKT cells [17, 100, 106]. IL-21 also augments proliferation of T cells stimulated via the TCR [106]. The synergistic stimulatory effect of IL-21 and IL-15 on CD8+ T cells permits clonal expansion of adoptively transferred tumor antigen-specific CTLs and promote tumor regression upon stimulation of these cells by the tumor antigen [68, 171]. Recent studies from our laboratory has shown that IL-7 is more potent than IL-15 in stimulating naïve CD8+ T cells in synergy with IL-21 [35].

Contact with DCs has been shown to facilitate antigen-independent proliferation of naive and memory CD4+ T cells. DCs can form stable conjugates with naive T cells and stimulate proliferation and effector functions in T cells in the absence of nominal antigen [24, 70, 119]. In a DC-naïve T cell co-culture system, antigen-independent proliferation of naïve T cells requires soluble factors secreted by DCs [41]. One of these soluble factors could be IL-15 because inflammatory stimuli upregulate IL-15 expression in DCs [87] and DC-derived IL-15 potentiates delayed-type hypersensitivity reactions [127]. Interestingly, IL-15Rα recycles and presents IL-15 to adjacent cells [26]. Hence it is likely that that DC-derived IL-15, presented by IL-15Rα, may potentiate DC-T cell interaction and deliver a signal strong enough to initiate antigen non-specific activation of T cells [115].

SIGNALING MECHANISMS UNDERLYING ANTIGEN NON-SPECIFIC ACTIVATION OF NAÏVE CD8+ T CELLS

Receptors for IL-7, IL-15 and IL-21 contain distinct ligand-binding α subunits and the common γ chain (γ_c) [71]. IL-2 receptor (IL-2R) and IL-15R complexes also share the IL-2/15Rβ subunit. These cytokine receptors rely on the JAK-STAT signaling pathway to transduce cellular activation signals [55]. Whereas IL-2, IL-7 and IL-15 selectively activate STAT5, IL-21 induces strong STAT3 activation and a less-pronounced activation of STAT1 [36, 53, 71, 76]. Among the cytokines that promote T cell activation or survival [85, 152], IL-6 induces a closely similar activation of STAT3 and STAT1 as IL--21 [49]. Unlike the γ_c cytokines, IL-6 utilizes the gp130 receptor subunit for signaling. Both IL-6 and IL-21 have been reported enhance the viability of T cells [123, 146, 152, 171] and synergize with TCR signals to augment T cell proliferation [106, 137]. In line with these signaling and functional similarities, we have recently shown that IL-6 can substitute for IL-21 in stimulating antigen non-specific activation of naïve CD8+ T cells in the absence of specific TCR stimulation [35].

Antigen non-specific proliferation of naïve CD8+ T cells induced by combinations of cytokines (IL-7 or IL-15 in presence of IL-21 or IL-6) correlates with increased STAT5 phosphorylation and its DNA-binding activity [35]. In naïve CD8+ T cells, IL-7 induces strong STAT5 phosphorylation whereas IL-15 elicits a weak

phospho-STAT5 signal. Concomitant stimulation with IL-6 or IL-21 significantly augments STAT5 phosphorylation induced by IL-7 or IL-15, whereas neither IL-7 nor IL-15 causes an appreciable increase in STAT3 phosphorylation induced by IL-6 or IL-21 [35]. The increased STAT5 signaling could be an important mechanism underling the proliferation of $CD8^+$ T cells stimulated by IL-7 or IL-15 in synergy with IL-21 or IL-6, and is consistent with the observation that transgenic expression of a constitutively active form of STAT5 causes accumulation of $CD8^+$ T cells [10]. Similarly, intensifying the IL-2-induced STAT5 activation by extending the half-life of IL-2 using anti-IL-2 antibody can stimulate naïve $CD8^+$ T cells *in vivo* [63]. In addition to the STAT signaling, Src kinases, PI3K and p38 MAPK are also implicated in antigen non-specific proliferation of naïve $CD8^+$ T cells induced by cytokines [35, 170]. Whereas activation of PI3K is a critical survival pathway stimulated by IL-7 [60], the roles of p38MAPK and Src kinases in cytokine-driven activation of $CD8^+$ T cells need to be elucidated. Similarly, it remains to be addressed whether the TCR is dispensable for antigen non-specific activation of naïve $CD8^+$ T cells by cytokines.

Signaling via cytokine receptors is regulated by several regulatory mechanisms to maintain cellular homeostasis [169]. Members of the "suppressor of cytokine signaling" (SOCS) family of proteins are important regulators of cytokine signaling [57]. SOCS1 gene is rapidly induced by several γ_c cytokines in $CD8^+$ T cells. Mice lacking SOCS1 generate and accumulate $CD8^+$ $CD44^{hi}$ "memory phenotype" cells, which respond robustly to IL-15 [56,112]. By regulating IL-15 and IL-21 signaling, SOCS1 might control antigen-independent activation of naïve $CD8^+$ T cells mediated by inflammatory cytokines [36] in order to prevent clonal expansion of potentially autoreactive $CD8^+$ T cells, discussed later in this review.

PRIMING OF NAIVE $CD8^+$ T CELLS BY CYTOKINES: IMPLICATIONS FOR ADAPTIVE IMMUNE RESPONSE

The efficiency of antigen-specific $CD8^+$ T cell response is markedly influenced by cytokines. In fact, after a brief encounter with antigenic peptide, proliferation of activated $CD8^+$ T cells can be sustained by IL-2 alone, which can be further augmented by IL-7 or IL-15 [167]. Apart from the γ_c cytokines IL-2, IL-4, IL-7 and IL-15, other cytokines such as IFN-α, IFN-γ, IL-6, IL-12, and IL-21 have also been implicated in potentiating $CD8^+$ T cell responses *in vitro* and *in vivo* [12, 69, 85, 137, 160, 166]. Whereas IL-2, IL-4, IL-7 and IL-15 could function by promoting cell survival via induction of anti-apoptotic proteins, IFN-α does not induce Bcl-2 or Bcl-xL but upregulates several proliferation-associated genes [85, 86, 160]. Among the cytokines that promote $CD8^+$ T cell response, IL-12, IFN-α and IL-21 provide a "third signal", which is distinct from the stimulatory signals delivered via the TCR, costimulatory receptors or the IL-2R (Fig. 1). This third signal promotes the survival of activated $CD8^+$ T cells, however the molecular nature of this signal remains to be elucidated [18, 19, 47, 69, 91].

It has been shown two decades ago that IL-6, in synergy with IL-1, could induce CTL responses on murine $CD8^+$ T cells [102, 117]. IL-6 was shown to act prior to IL-1 by augmenting IL-2 responsiveness of $CD8^+$ T cells in mixed lymphocyte cultures, suggesting a potential role for IL-6 during the early phase of $CD8^+$ T cell response [102]. In support of such stimulatory function for IL-6 in CTL responses, we have recently shown that IL-6 can prime $CD8^+$ T cells for subsequent stimulation via the TCR [35]. Naïve P14 TCR transgenic $CD8^+$ T cells that have been previously exposed to IL-21 or IL-6 in the presence or absence of IL-7 or IL-21 display increased sensitivity to antigen resulting from a reduction in their threshold for stimulation via the TCR. These cytokine-primed T cells also develop more potent lytic functions towards target cells. Further studies are needed to understand the molecular mechanisms underlying the priming function of IL-6 and IL-21 on naïve $CD8^+$ T cells.

During immune response to microbial pathogens, neutrophil-mediated acute inflammation precedes the monocyte- and lymphocyte-dependent adaptive immune response [54, 61, 64]. The ability of inflammatory cytokines (IL-6 and IL-21) to synergize with cytokines that regulate T cell homeostasis (IL-7 and IL-15) could play a critical role during this transition leading to stimulation of naïve $CD8^+$ T cells. Whereas IL-6 is produced by several cell types including neutrophils, DCs, macrophages, $CD4^+$ T cells and $CD8^+$ T cells, IL-21 is produced by NKT cells and activated $CD4^+$ T cells [17, 99, 106]. Hence, IL-6 and IL-21 will be available from the very beginning of an immune response as effector molecules of the innate immune system. Myeloid cells also produce IL-15 in response to innate immune stimuli [144]. Hence, it is conceivable that the combined action of inflammatory cytokines could lead to local activation of recirculating $CD8^+$ T cells at inflammatory sites (Fig. 1), facilitating their subsequent stimulation by cognate antigens in draining LNs.

The distinct cell surface phenotype of cytokine-stimulated cells may also contribute to the effectiveness of the adaptive immune response. Unlike TCR stimulation, priming of $CD8^+$ T cells by the synergistic cytokine stimulation augments the expression of CD62L [35, 51], which would facilitate the cytokine-primed $CD8^+$ T cells to re-enter peripheral LNs in search of specific antigens [37, 38]. In fact, an important function for IL-6 in the transition from innate to adaptive immune response seems to be to promote migration of T cells to the inflammatory sites [90, 164]. Phenotypic changes associated with the IL-6-induced migratory capacity needs further investigations.

CYTOKINE-MEDIATED ACTIVATION OF CD8+ T CELLS: POTENTIAL CONTRIBUTION TO AUTOIMMUNITY

While antigen non-specific activation of CD8+ T cells by inflammatory cytokines could be beneficial to mount protective immune responses, it can also contribute to perpetuation of chronic inflammatory conditions and progression of autoimmune disease [58, 61, 64]. CD8+ T cells significantly contribute to several autoimmune diseases including type 1 diabetes (T1D), systemic lupus erythematosis, multiple sclerosis, inflammatory bowel disease and arthritis [163]. Autoimmune destruction of target tissues is often preceded by and associated with inflammatory conditions that could provide an ideal milieu for antigen non-specific activation of CD8+ T cells. Since autoreactive T cells bear low affinity TCRs towards tissue antigens, diminishing the threshold level of stimulation required for triggering TCR signaling by prior cytokine stimulation [35] would facilitate clonal expansion of potentially autoreactive CD8+ T cells. Furthermore, increased cytolytic potential of cytokine-primed CD8+ T cells [35] could also contribute to the pathogenesis of autoimmune diseases.

It has long been suggested that cytokines that regulate homeostasis of CD8+ T cells may promote expansion of autoreactive CD8+ T cells [30, 73]. Indeed, homeostatic proliferation has been associated with autoimmunity in experimentally-induced lymphopenia [44]. In Biobreeding diabetes-prone strain of rats and in the NOD mouse, naturally occurring lymphopenia is an important underlying cause for the development of T1D [39, 113]. In case of T1D in the NOD mouse, IL-21 signaling has been associated with the activation of autoreactive CD8+ T cells [67]. IL-15 has long been implicated in rheumatoid arthritis, though IL-15 also influences many other cell types [89]. Dysregulated IL--15 signaling arising from SOCS1 deficiency can activate autoreactive CD8+ T cells in an experimental model of T1D [22]. Recent studies have implicated IL-7 in autoimmune diseases [11, 78]. IL-6 has long been associated with inflammation and autoimmunity [58, 131]. In an experimental model of colitis, IL-6-driven proliferation and IL-17 production by CD8+ T cells underlie pathogenesis [131, 145]. Transient abundance of cytokines that can trigger antigen non-specific activation of CD8+ T cells can also occur in normal animals during microbial infections. For example, viral infections not only increase the abundance of IL-7 by reducing its utilization as a consequence of lymphopenia [107, 109] but also induce *de novo* expression of IL-15 [172]. These considerations lend support to the notion that cytokine-driven bystander activation of potentially autoreactive CD8+ T cells could occur during viral infections [162]. In fact, cytokine-driven non-specific activation of CD8+ T cells could be the common denominator for the seemingly unexplainable association of a wide variety of apparently unrelated pathogens with a particular autoimmune disease [62].

USE OF CYTOKINES TO GENERATE TUMOR-SPECIFIC CTLs FOR ADOPTIVE CELL THERAPY

CD8+ CTLs are the key effector cells of anti-tumor immunity [124, 138]. Anti-tumor CTLs are activated via the TCR by antigenic peptides that are generated from tumor-associated antigens (TAAs) by proteasomes and presented by MHC class I molecules [118]. In addition to stimulation via the TCR, signaling via costimulatory receptors is essential to initiate a productive CTL response [72, 88]. Most mammalian cells express MHC-I but lack the co-stimulatory molecules [104]. Hence, induction of an efficient CTL response requires the help of professional antigen presenting cells such as mature DCs [120]. DCs engulf fragments of dead tumor cells, migrate to LNs, cross-present tumor-derived antigenic peptides to CD8+ T cells along with costimulatory signals [4, 101]. Activated CTLs kill tumors via perforin- and Fas- mediated pathways [108, 156].

Stimulation of efficient anti-tumor CTL response is the cornerstone of cancer immunotherapy. Identification of large number of TAAs stimulated efforts to induce highly specific anti-tumor response [118]. However, vaccination against TAAs has been largely ineffective due to failure to induce high-avidity CTL response and tolerization of the antigen-specific CTLs [125, 173]. It has been well established that the tumor microenvironment is highly immunosuppressive that impedes the initiation and execution of an efficient CTL response [33, 93, 141, 173]. Several tumor-derived factors such as soluble Fas ligand, prostaglandins, IL-10, IL-13 and TGF-β can inhibit CTL responses by blocking DC maturation, inducing regulatory T cells, and by inhibiting the activation and functions of CTLs [173]. To overcome some of these limitations, DCs that are generated *in vitro*, modified to improve antigen presenting functions and pulsed with tumor antigenic peptides or tumor cell lysates are being intensely explored as novel cancer vaccines [4, 32, 133, 134].

Administration of IL-2 to promote expansion of inefficiently activated and possibly anergized tumor-specific T cells showed a significant but limited success in causing tumor regression in cancer patients [126]. Nonetheless, this approach clearly demonstrated the potential benefits of manipulating the immune system against human cancers and has prompted the use of other stimulatory cytokines, and antibodies against negative regulatory receptors such as CTLA-4 and PD-1 to boost anti-tumor immune responses in human and experimental animal models [52, 111, 124]. In parallel, *ex vivo* expansion of tumor-infiltrating lymphocytes (TILs) from the tumor mass to generate tumor-specific CTLs for adoptive cell therapy (ACT) has emerged as a very promising approach for cancer immunotherapy [124]. ACT offers many advantages over cytokine therapy or vaccination against human cancers: ACT 1) circumvents the immunosuppressive effects of tumor burden on the initiation of anti-tumor CTL response *in*

vivo; 2) diminishes the possibility of inducing autoreactive CTLs, a known deleterious effect of cytokine therapy [13, 27, 80]; 3) can incorporate strategies to generate high avidity CTLs and target them to tumors [97, 128]; and 4) can be combined with lymphodepletion and cancer chemotherapy, which potentiate ACT by eliminating regulatory T cells that suppress the immune response, by generating "space" for the expansion of adoptively transferred tumor-specific CTLs and by improving antigen presentation [28, 74, 124].

Ex vivo expansion of tumor-infiltrating CD8+ T cells in the presence of IL-2 needs further optimization to generate tumor-specific CTLs for ACT. Even though the expanded CTLs were selected based on their ability to secrete effector cytokines such as IFN-γ and to kill tumor targets *in vitro*, these parameters do not truly reflect their *in vivo* clinical efficacy [40]. Importantly, evaluation of transgenic mouse CD8+ T cells specific to a tumor antigen, at different stages of their differentiation following *in vitro* stimulation by antigen and IL-2, has revealed that acquisition of full effector functions impaired their ability to kill tumor cells *in vivo* [40]. Hence, the clinical outcome of ACT using tumor-specific CTLs generated *ex vivo* depends not only on the cell yield but also on several other parameters such as their avidity towards tumor antigens, expression of effector functions, proliferation potential, migration properties and longevity in the recipients [40, 121, 124].

Recent studies have shown that IL-21 can enhance anti-tumor immunity via its stimulatory effects on NK cells and CD8+ T cells [139]. Particularly, in combination with IL-15, IL-21 stimulates proliferation and effector functions of CD8+ T cells and generates tumor-specific CTLs with enhanced antigen reactivity and effector functions [36, 171]. CD8+ T cells expanded by IL-15 and IL-21 display a distinct phenotype with elevated expression of CD62L that could have important implications for their recirculation in search of specific antigen [35, 51]. Furthermore, unlike IL-2, IL-21 can stimulate naïve CD8+ T cells in the presence of IL-7 [36, 171]. Hence, by using this cytokine combination to stimulate TILs *in vitro*, it might be possible to activate naïve tumor-specific CD8+ T cells that might have been suppressed *in vivo* by the tumor microenvironment. Currently, the efficacy of ACT is limited to a few types of solid tumors. By improving the methods used for generating highly effective tumor-specific CTLs, it would be possible to employ ACT for personalized treatment of other types of cancers as well.

CONCLUSIONS

Antigen non-specific activation of T cells by cytokines during conditions of lymphopenia has probably evolved as a mechanism to rapidly reconstitute the T cell compartment. Cytokines that govern this homeostatic proliferation can become available in abundance during conditions of infection and inflammation. *In vitro* studies suggest that antigen non-specific activation of CD8+ T cells may have a beneficial role during infections by promoting proliferation and effector functions of antigen-specific naïve T cells. On the other hand, inadvertent activation of potentially autoreactive CD8+ T cells may lead to autoimmunity. Specific immunotherapeutic agents directed at nullifying the stimulatory effects of IL-7, IL-15, IL-6 and IL-21 may be useful in reducing the severity of autoimmune diseases in which CD8+ T cells play an important role in pathogenesis. On the other hand, use of these cytokines to expand tumor specific CD8+ T cells *in vitro* has been emerging as a promising strategy for cancer immunotherapy.

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