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Cytolytic CD4 Cells: Direct Mediators in Infectious Disease and Malignancy

Deborah M. Brown
University of Nebraska-Lincoln, School of Biological Sciences and Nebraska Center for Virology, 142 Morrison Life Sciences Research Center, 4240 Fair St., Lincoln, NE 68583-0900

Deborah M. Brown: dbrown7@unl.edu

Abstract

CD4 T cells have traditionally been regarded as helpers and regulators of adaptive immune responses; however, a novel role for CD4 T cells as direct mediators of protection against viral infections has emerged. CD4 T cells with cytolytic potential have been described for almost forty years, but their role in host protection against infectious disease is only beginning to be realized. In this review, we describe the current literature identifying these cells in patients with various infections, mouse models of viral infection and our own work investigating the development of cytolytic CD4 cells in vivo and in vitro. CD4 CTL are no longer considered an artefact of cell culture and may play a physiological role in viral infections such as EBV, CMV, HIV and influenza. Therefore, vaccine strategies aimed at targeting CD4 CTL should be developed in conjunction with vaccines incorporating B cell and CD8 CTL epitopes.

Introduction

T lymphocytes are subdivided based on recognition and response to antigen: CD8 T cells recognize peptides of approximately 8-10 amino acids within Major Histocompatibility (MHC) class I proteins while CD4 T cells recognize peptides of approximately 12-15 amino acids in the context of MHC class II molecules. There also exists a functional dichotomy in adaptive immune responsessuch that, CD8 T cells mediate pathogen clearance by active killing of infected host cells, while CD4 T cellssecrete cytokines aiding in the differentiation of B cells to plasma cells and maintaining memory CD8 responses. Indeed, the role of CD4 T cells in the induction and maintenance of adaptive immunity appears to be indirect and includes orchestration of B cell responses, macrophage activation, CD8 memory generation and downregulation of responses after pathogen clearance[1]. CD4 T cells are also further divided into subsets based on cytokines produced and protection against different types of pathogens. For example, Th1 cells secrete IFN-γ and are important against viral and intracellular bacterial infection while Th2 cells secrete IL-4 and IL-5 and provide protection against extracellular parasites [1]. Another subset, termed Th17, has also been described recently that produces IL-17, is important against fungal infections, regulates inflammation during infection and can promote autoimmunity [2]. Finally, T regulatory cells (Treg) are also part of the CD4 T cell lineage and these cells act to maintain peripheral tolerance and downregulateresponses after infection. The development of each subset is controlled by a unique transcription factor and

once the developmental program is established, genes that promote the other subsets are silenced.

In addition to the helper functions traditionally assigned to the CD4 T cell subset, a more direct role for CD4 T cells in cell-mediated immunity has recently been appreciated. Although CD4 T cells with cytolytic potential have been described for decades, current data has emerged suggesting that class II restricted CD4 CTL contribute to protective responses against viral and bacterial infections as well as tumor responses. This review will examine the historical data describing CD4 CTL, the models used to study thegeneration and regulation of CD4 CTL, their in vivo relevance and the clinical importance of these cells as vaccine targets or therapeutics.

CD4 T cellswith cytotoxic potential

Class II restricted CD4 effectors with cytolytic potential have been described since the late 1970's[3]. Stimulation of these cells was accomplished with the mixed lymphocyte reaction, an alloreactive response that induces a strong signal in up to 5% of all T cells. Later reports demonstrated that CD4 CTL could be generated in T cell clones that were reactive to influenza [4], poliovirus[5], Epstein Barr virus[6], measles virus [7], and herpes simplex virus[8], suggesting that these cells develop against viral antigens. The appearance of cytolytic CD4 cells during primary infection has also been documented but these cells were identified in mice that lacked the normal complement of CD8 T cells [9;10]. Taken together, many early reports labeled CD4 CTL as an in vitro artefact and there was speculation whether these cells had any in vivo relevance.

More recently, CD4 cells have been identified in peripheral blood of subjects exposed to CMV [11], EBV[12], and HIV [13] and in mice infected with murinegammaherpesvirus [14]. These circulating CD4 cells appear to be terminally differentiated and are hypothesized to be generated by chronic exposure to antigen[15]. Work with CD4 T cell clones would seem to support this since those cells have been repeatedly stimulated in vitro. However, CD4 CTL have been described in immunocompetent mice infected withLCMV Armstrong strain[16], and influenza (Brown and Swain, submitted), indicating that CD4 CTL can be generated in acute infections. In fact, our work also shows that cytolytic activity in CD4 cells can be generated after just three days in culture with a single primary stimulation [17]. These results indicate that cytolytic CD4 cells arise duringboth chronic and acute infection and may be important against pathogens that evade the classical class I processing pathway.

Two major mechanisms of cell killing have been described for cells of the immune system. One involves binding of a cell surface receptor known as Fas on T cells with Fasligand (FasL) on the target cell. The other mechanism used by CTL is granule exocytotosis whereby perforin and granzyme are secreted by T cells after recognition of peptide antigenin MHC[18]. Both the Fas:FasL and perforin/granzyme pathways culminate in activation of caspases and induction of apoptosis in target cells[19]. The Fas:FasL mediated cytotoxic pathway is believed to be the main pathway of lytic activity by CD4 T cells and is implicated in the downregulation of immune responses. Antigen presenting B cells express high levels of FasL on their surface and are especially sensitive to the Fas mediated pathway of cell death by cytolytic CD4 cells [20]. CD4 CTL have also been shown to lyse A20 mouse B cell lymphomas [21] and human B cell lymphomas transformed by EBV infection [6]. Other reports provide evidence that CD4 T cells can use perforin and granzyme B (grB) [22;23;24;25], and human CD4 CTL can utilize granulysin[26;27;28]. Granulysin is not expressed in mice and appears to be important in mycobacterial[26;27]and fungal infections [29] in humans. Perforin and grB mediated cytotoxicity by CD4 CTL has been shown to be involved in responses against HIV [13], CMV [11;13], EBV [23], HSV [30], influenza [22], and against B cell lymphocytic leukemia [31]. Therefore, CD4 CTL can use a variety of mechanisms to induce killing of target cells and most

of these pathways are not mutually exclusive. Our own data demonstrates that CD4 CTL primarily utilize perforin to lyse target cells, but when IL-2 is limiting, CD4 cells can also use Fas:FasL[17].

A more direct role for CD4 T cell in infectious disease and malignancy

Much of the early work using CD4 T cell clones generated against viral antigens used these cells to demonstrate the ability of CD4 cells to be protective against lethal challenge with virus. Transfer of CD4 CTL prior to inoculation with lethal dose of virus protected mice against influenza infection[4;22] poliovirus [5] and West Nile virus [32]. In these reports, CD4 cells mediated protection directly, in the absence of CD8 or B cells, or before the primary response in normal mice could develop. Similarly, CD4 T cell clones from patients harboring EBV, can directly lyse EBV infected B cells, or transformed B cellsexpressing EBV proteins [12;28; 33]. There are also reports of humancytolytic CD4 cells being generated in culture and adoptively transferred to patients with malignancy[34;35]or in stem cell recipients infected with EBV or CMV[36]. These reports indicate that CD4 CTL have a direct role in protection against lethal virus infection and may be therapeutic against certain malignancies. One hurdle in understanding the nature of protection in these instances is the availability of class II expressing targets in many of these infections. Class II expressing B cells are latently infected with EBVandlysis of these cells can occur any time during viral protein expression or during transformation. In addition, LCMV can infect cells of the immune system, also providing class II expressing targets for CD4 CTL. However, many infections such as CMV and influenza target cells that normally do not express class II molecules, so the role of cytolytic CD4 cells in these infections may be more obscure. It has been demonstrated thatMycobacterium tuberculosis [37], andparainfluenza[38]infection, or treatment with IFN-γ[39]increase the level of class II expression on lung epithelial cells and possibly provide targets for cytolytic CD4 cells. Our results suggest that class II is upregulated on type II epithelial cells after influenza infection and transferred cytolytic CD4 cells are in close proximity to the epithelial layer (Brown and Swain, submitted). Furthermore, we have shown that transferred CD4 CTL can decrease viral titers in the lung four days post lethal infection, before CD8 cells migrate to the lung [22]. Therefore, many infections inducean inflammatory milieu, upregulate class II expression on epithelial andendothelial cells and provide targets for CD4 CTL lysis.

As mentioned previously, CD4 CTL may downregulate immune responses by lysing antigen presenting B cells via Fas:FasL interactions[20]. CD8 CTL also modulate immune responses by eliminating dendritic cells, thus limiting antigen presentation during priming [40;41]. We do not believe that CD4 CTL modulate APC function in the lymph node during priming after influenza infection since CD4 cells isolated from the draining LN demonstrate low grB expression and lackcytolytic activity. Immunofluorescent studies in influenza infected lungs reveal CD4 CTL in proximity to class II expressing epithelial cells and clustered with class II expressing cells in the lung parenchyma that may be macrophages or dendritic cells (Brown and Swain unpublished observation). While we have yet to definitively identify the class II expressing cell in the lung parenchyma, we speculate thatCD4 CTL may act to downregulate APC populations in the lung as virus is being cleared.

Models of cytolytic CD4 generation and differentiation

While many reports correlate CD4 CTL generation with chronic infection and terminal differentiation state [15;42], there is a paucity of literature describing the factors that promote and regulate cytolytic activity in developing CD4 effectors. We use a T cell receptor (TCR) transgenic (Tg) model in which all CD4 T cells recognize a peptide from hemagglutinin protein in influenza virus PR8. We have developed an in vivo model in which TCR Tg cells are adoptively transferred to normal, immunocompetent mice, followed by sublethal infection with

influenza PR8[43;44;45]. Using this model we can investigate the CD4 T cell specific cytolytic response to acute infection with influenza (Brown and Swain, submitted). We have also use these antigen specific CD4 T cells in an in vitro system where naïve T cells are cultured with peptide pulsed antigen presenting cells and various cytokines to determine which factors are required for generation of the cytolytic phenotype [17].

In vivo models

In humans, surface marker analysis of CD4 CTL reveals a circulating cell that is $CD45RO^+$, $CCR7^-$, $CD27^-$ and $CD28^-$[13;46]. Casazza, et al. also correlates $CD57^+$ expression with high levels of granzyme A (grA), grB and perforin[11]. These cells can be isolated from peripheral blood and shown to be cytotoxic directly ex vivo, suggesting that these cells are antigen experienced, terminally differentiated effectors. Recently, in the mouse model of persistent gammaherpes infection, a population of $CD27^-$CD4 cells in the spleen and lung was found to be cytolytic against gHV68 infected targets directly ex vivo and in vivo suggesting that highly differentiated CD4 cells can control this infection by direct cytotoxicity[14]. Our in vivo model confirms these findings as we have demonstrated that CD4 cells isolated from the lung during influenza infection are highly cytolytic against peptide pulsed targets ex vivo. This correlates with acquisition of IFN-γ and loss of IL-2 secretion in the lung as well as loss of CD62L, partial loss of CD27 and increased CD43 expression (Brown and Swain, submitted). Our studies are unique in that we have demonstrated acquisition of cytolytic activity in CD4 cells in vivo in response to an acute infection, rather than a chronic infection. In addition, $CD4^+grB^+$ cells are both $CD27^+$ and CD27 in the lungsuggesting that chronic infectionsuch as gammaherpes virus may further differentiate CD4 CTL to $CD27^-$ cells while acute infection (influenza) induces a less differentiated cell that is CD27 (Figure 1). It remains to be determined whether both $CD27^+$ and $CD27^-$ populations can lyse targets ex vivo, or whether grB expression precedes lytic ability and only the $CD27^-$ population is effective killers. Infection of mice with influenza also provides us with a model of in vivo CD4 CTL differentiation since influenza specific CD4 cells from the draining lymph node (dLN) do not lyse peptide pulsed targets while CD4 cells isolated from the lung exhibit high levels of lysis. This correlates with IL-2 secretion in the dLN, followed by switching to IFN-γ secretion in the lung. It remains to be determined whether CD4 CTL receive additional signals in the lung to differentiate to cytolytic cells, or whether they acquire this activity while in transit from the dLN to the site of infection. In addition, it is not known whether the same CD4 cell can act as a “helper” cell in the dLN, then acquire cytolytic and migratory capabilities to become CTL in the lung, or whether these two functionally different cells represent unique subsets. We are actively pursuing these separate hypotheses in our in vivo model of CD4 CTL differentiation.

In vitro models

Using the in vitro model, we have confirmed what has previously been described for CD4 T cell clones, in that, Th1 polarization correlates with cytotoxicity and protection against influenza [4;22;47], poliovirus [5], EBV [6;33] and West Nile virus [32]. In contrast, Th2 polarized effectors did not demonstrate efficient lysisand IL-4 was shown to inhibit the generation of CD4 CTL when increasing concentrations were added to culture conditions [17]. Furthermore, Th2 effectors were less able to protect mice against lethal influenza infection (Brown and Swain, unpublished observations).

Our in vitro system provides an excellent model with which to define the parameters necessary to drive the differentiation of naïve CD4 T cells to the cytolytic phenotype. Not only do Th1 polarized CD4 effectors exhibit lytic activity, but naïve CD4 cells activated in the presence of antigen and IL-2 alone (Th0) acquire grB expression and perforin mediated cytotoxicity. When compared with Th1, Th2 and Th17 polarized effectors, cells activated with IL-2 only express both Th1 and Th2 type cytokines including IFN-γ and IL-4, and retain the ability to secrete

IL-2 [17]. These data suggest that cytolytic cells can be generated without polarizing signals and may represent a less differentiated cell than data obtained with human subjects would indicate (Figure 2). Although Th0 cells can be generated in vitro, demonstrate the highest level of cytotoxicity and correlate with a less differentiated phenotype, CD4 CTL generated in vivo tend to be more differentiated due to Th1 polarizing cytokines. In addition, in vivo generated CD4 CTL are $CD62L^-$, $CD43^+$ and $CD27^{+/-}$, while a proportion of in vitro generated CD4 effectors are $CD62L^+$ and express moderate levels of CD43 and CD25 [17] and (Brown and Swain, submitted). Furthermore, we have identified a hierarchy in cytolytic activity amongst CD4 T cell subsets with Th0 ≥ Th1 > Th2 > Th17 ([17] and (Moore and Brown, in preparation)). What remains to be determined is whether there is plasticity in these CD4 cell subsets, or whether secondary stimulation with antigen and IL-2 in vitro can induce cytotoxicity in previously polarized subsets.

Using this in vitro model we have also determined that antigen presenting cells (APC) were absolutely required for the generation of cytolytic CD4 cells and that concentrations of anti-CD3 and anti-CD28 used to activate canonical helper CD4 T cells did NOT induce peptide specific killing[17]. This is in contrast to results obtained from groups using both polyclonal [48]and TCR Tg systems [49]in which CD8 cells activated with anti-CD3/anti-CD28 can lyse target cells effectively. Because our system requires peptide pulsed APC, we attempted to determine whether APC derived cytokines were playing a role in driving CD4 CTL generation. We ruled out IL-12, IL-10, TGF-β, TNF-α and IL-6 using either blocking antibodies or APC deficient in these cytokines in either enhancing or inhibiting CTL activity in CD4 T cells. Our recent work has demonstrated that bone marrow derived dendritic cells (BMDC) are more effective at inducing cytolytic activity when compared to LPS activated B cell blasts or A20 B lymphoma cells. The hierarchy of lytic ability in CD4 T cell subsets remains: Th0 ≥ Th1 > Th2 > Th17, however, Th17 cells show moderate cytotoxicity when generated with BMDC compared to no cytotoxicity when activated with B cell blasts (Moore and Brown, in preparation). Cytokine blocking experiments with BMDC as APC must be repeated in this system and we hypothesize that IL-1 may play a role due to its ability to enhance T cell activation [50;51]and our observation that BMDC can secrete high amounts of this cytokine compared to B cell blasts.

It remains to be determined whether CD4 CTL represent a terminal differentiation state in Th1 development or whether CD4 CTL are a unique CD4 T cell subset with distinct cellular and molecular requirements for differentiation. How CD4 CTL relate to canonical helper CD4 cells or follicular helper cells are questions we can begin to answer using these in vitro and in vivo models of differentiation. It is clear that CD4 CTL are important in many viral infections whether providing an extra layer of protection in acute infection, or providing primary protection against viruses that evade the immune response by down-regulating class I presentation.

Molecular mechanisms of CD4 CTL differentiation

Many reports have documented the ability of CD4 T cells to lyse class II expressing targets using cell surface expression of FasL binding to Fas expressed on target cells [10;20;21]. Our lab and others have shown that CD4 T cells can use grB and perforin to lyse target cells directly ex vivo [22;23;24;25]. CD4 TCR Tg cells that lack perforin, do not lyse peptide expressing targets after sublethalinfluenza infection indicating that the granule exocytosis pathway is the major cytolytic pathway used by CD4 T cells to lyse targets in vivo. This is similar to data identifying cytolytic CD4 cells in peripheral blood that expressgrB and perforinafter infection [11;13;42]. Surprisingly, our in vitro model demonstrates that CD4 cells can lyse targets by both FasL- and perforin-mediated mechanisms that aredependent upon peptide dose during effector generation. High levels of peptide, in the 5 μM range, induces 5-10 fold expansion in

CD4 cells, high IFN-γ secretion and moderate cytolytic activity that is partially blocked by antibodies to FasL. In contrast, lower amounts of peptide (5-50 nM) induce much less expansion, more Th2 cytokines and high levels of cytolytic activity [17]. This would seem to contradict our earlier finding that Th1 cells have a higher cytolytic activity than Th2 cells, however, it appears that cytokines present early in the priming of naïve CD4 T cells is what dictates whether a naïve CD4 T cell will acquire cytolytic activity. For example, IL-4 present early in the culture will inhibit CD4 CTL generation while IL-12 seems to have no effect. The phenotype of the CD4 CTL, at least in vitro, appears to be a multifunctional cell that can secrete IFN-γ, IL-2, some IL-4 and IL-5 along with cytolytic activity (Figure 2). We have also demonstrated that addition of exogenous IL-2 is required to induce perforin mediated cytotoxicity, especially at low peptide doses, while FasL mediated cytotoxicity can be induced without IL-2 [17].

We are beginning to elucidate the molecular mechanisms involved in IL-2 induced, perforin mediated killing in CD4 T cells. When IL-2 binds to the IL-2 receptor complex, januskinase (JAK) 1 and 3 are activated which in turn phosphorylates the signal transducer and transactivator (STAT) 5. STAT5 can then bind to the perforin gene and upregulate expression [52]. Using CD8 and CD4 human T cell lines, it has been shown that perforin expression is differentially regulated in CD8 vs CD4 T cells. CD8 T cell clones showed increased binding of STAT5 and expressed high levels of perforin in both the resting and activated state while cytolytic CD4 clones demonstrated STAT5 binding and expressed high levels of perforin only during activation [30]. Using a combination of IL-2 deficient, or IL-2Rα (CD25) deficient TCR Tg T cells we can determine the role of IL-2 and STAT5 in promoting perforin mediated cytotoxicity in CD4 T cells. Our preliminary work suggests that IL-2 needs to be present early during T cell priming, and that low (10 ng/ml) amounts of IL-2 are sufficient to upregulategrB and cytotoxicity. The lack of IL-2Rα diminishes cytotoxicity, but grB can still be upregulated in these cells suggesting that signaling through the IL-2 β chain or common γ chain may be sufficient for upregulation of granzyme, but signaling through the high affinity IL-2 receptor complex is necessary for complete cytolytic activity in CD4 T cells. Furthermore, IL-2 induces STAT5 phosphorylation that correlates with cytotoxicity. IL-7, a cytokine that shares the STAT5 signaling pathway as well as the common γ chain receptor also induces cytolytic activity in CD4 T cells, albeit, at lower levels compared to IL-2 (Canfield and Brown, in preparation). Studies are underway to determine whether STAT5is absolutely required for the generation of cytotoxicity in CD4 T cells and the role of other common γ chain cytokines are also being investigated.

Transcription factors have an important role in the development of CD4 T cell subsets, where unique factors simultaneously drive the differentiation of one subset while inhibiting the development of the other subset. It has been shown that the transcription factors T-bet, GATA-3 and RORγt, drive the differentiation of Th1, Th2 and Th17 cells respectively, by inducing cytokine synthesis in a positive feedback mechanism (reviewed in [1]. We hypothesize that CD4 CTL differentiation may be controlled by T-bet since Th1 cells demonstrate effective killing, however, we have also demonstrated that IFN-γ is not necessary for driving peptide specific cytolytic responses in CD4 T cells [17]. We speculate that the Th0 subset, those cells that are the most effective killers, will express T-bet as well as other factors are also be important in the generation of CD8 CTL [53].

Other transcription factors may also play a role in the differentiation of cytolytic CD4 cells, such as T-bet and eomesodermin (Eomes). These factors have been implicated in the differentiation of CD8 CTL since mice deficient in these proteins demonstrate lower cytolytic activity and decreased clearance of LCMV infection [54]. One report demonstrates that Eomes is not expressed in anti-CD3/CD28 activated CD4 cells [55], however, we have shown that this activation pathway does not induce effective antigen specific CD4 cytolytic activity [17].

Our method of inducing CD4 CTL with peptide pulsed APC may in fact induce Eomes, and those experiments are currently in progress. T-bet and Eomes also increase expression of IL-2R β in CD8 CTL, thus increasing responsiveness to IL-2 and IL-15 [56]. In addition, it has been shown that the Runx family of transcription factors is expressed in mature CD8 T cells and Runx-3$^{-/-}$cells demonstrate reduced cytotoxicity[57]. More recent data demonstrate that Runx3, T-bet and Eomes all cooperate to control the differentiation of cytolytic CD8 cells with T-bet involved in early regulation of IFN-γ, Eomes involved in late regulation of IFN-γ and perforin and Runx3 inducing expression of Eomes as well as perforin, grB and IFN-γ. We hypothesize that more than one transcription factor plays a role in the differentiation of CD4 CTL and studies are underway to determine the molecular profile that correlates with the Th0 subset and highly effective cytotoxicity.

Clinical Implications: vaccine design and immunotherapy

It is clear that CD4 T cells play pivotal roles in immune responses against infection and malignancy as well as pathological conditions such as asthma and autoimmunity. The effects of CD4 T cells during immune responses tend to be indirect, providing cytokine help for antibody production and maintenance of CD8 T cell responses. Cytolytic CD4 cells can exert direct functions against infections and malignancies by lysing class II expressing targets, therefore, vaccines strategies should aim to induce this subset of cells.

Current vaccine strategies for many infections such as influenza rely on generating neutralizing antibodies against the virus. However, outer viral proteins of influenza can mutate rapidly making the current vaccines ineffective and necessary to be reformulated every year. Incorporating both CD4 and CD8 T cell epitopes for many vaccines will help to induce cytolytic cell mediated immunity against many infectious diseases. For example, CD4 T cells are required for control of EBV infection and it has become apparent that much of this control is due to the cytolytic ability of CD4 T cells [14;33]. Recent vaccine strategies for EBV incorporated CD8 epitopes from LMP2 and CD4 epitopes from EBNA1 and were shown to reactivate both CD8 and CD4 cells in peripheral blood [58]. These strategies can also be applied for the control of EBV transformed malignancies such as Burkitt's lymphoma and certain nasopharyngeal carcinomas that express EBV proteins [14;33].

New adjuvants must also be developed to target viral and tumor antigens to dendritic cells (DC). Our work suggests that DC are important in inducing CD4 CTL, especially in vivo, and activation of DC with adjuvants that target toll like receptors (TLR) is a possible avenue of development[59]. Synthetic CpGoligonucleotide that targets TLR-9 has been documented to induce Th1 differentiation and provide protective responses against anthrax[60] and vaccinia [61]. Therefore, using adjuvants that enhance cellular immunity will allow the differentiation of cytolytic CD4 cells as well as cytokine secreting cells and increase the efficacy of protective responses against a number of infectious diseases. Indeed, one of the hallmarks of protective vaccination is the generation of a multifunctional CD4 cell that can secrete IL-2, TNF-α and IFN-γ[62]. Perhaps vaccination with Th1 inducing adjuvants can promote differentiation of a multifunctional cell with cytotoxic and cytokine secreting capabilities. Studies using TLR-3 and TLR-7 agonists as vaccines to induce CD4 CTL effectors and memory cells that can act in protection against lethal influenza are ongoing in our laboratory.

In conclusion, cytolytic CD4 T cells are important in many viral infections such as EBV, HIV and CMV and may be generated through repeated antigen stimulation in chronic infections. Mouse models of CD4 CTL generation indicate that these cells are induced by acute infection as well as chronic infections and can primarily utilize the granule exocytosis pathway as a mechanism of cell killing. There remains much to be learned about how these cells are generated, however, IL-2 and dendritic cells appear to be a major players in CD4 CTL

differentiation and vaccines designed to activate these pathways should be utilized for infectious diseases and malignancy.

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Abbreviations

APC	antigen presenting cell
BMDC	bone marrow derived dendritic cell
CMV	cytomegalovirus
EBV	Epstein Barr Virus
grB	granzyme B

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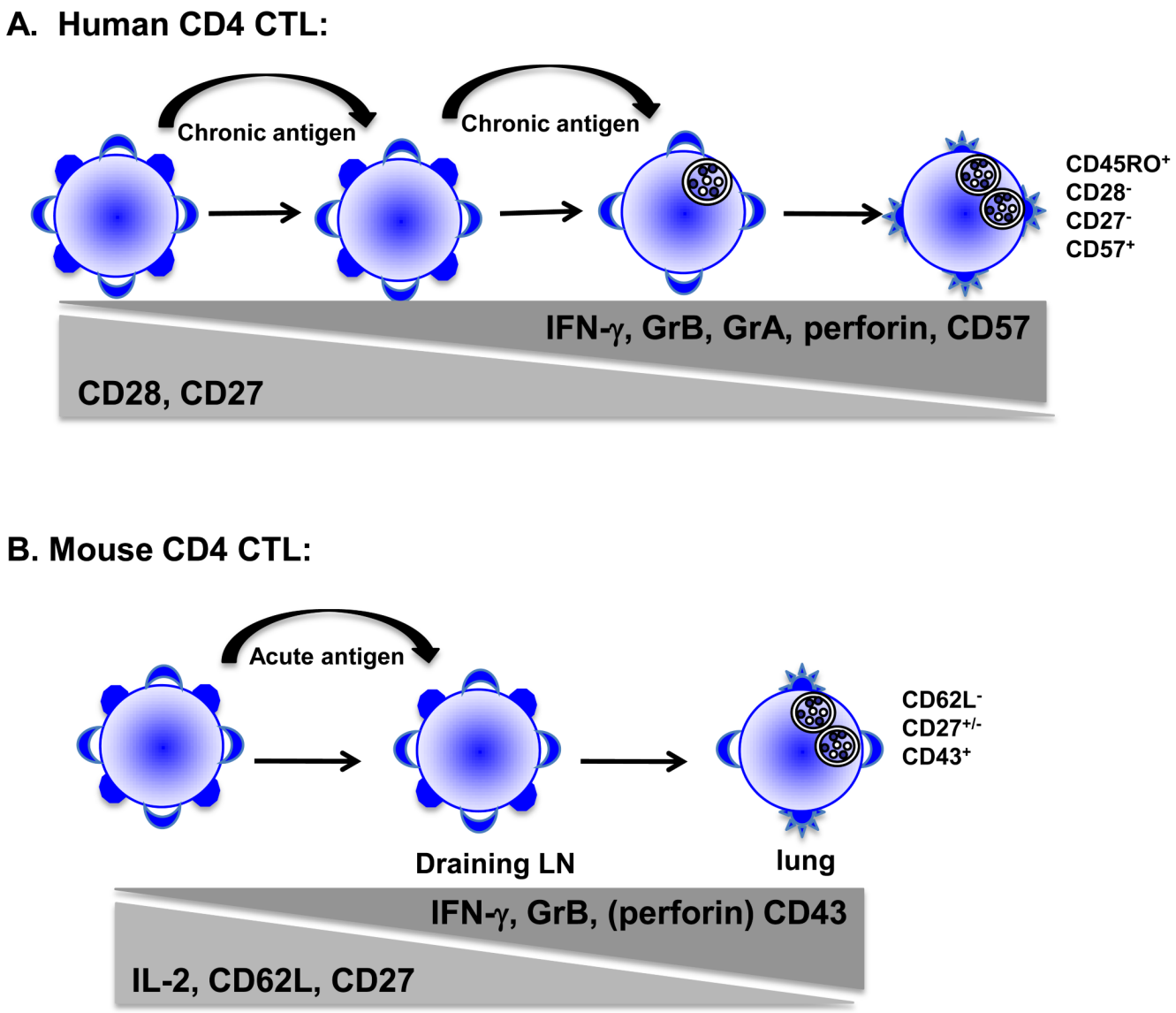

Figure 1.

A) Model of differentiation of human CD4 CTL. In many chronic infections induce a population of CD4 T cells with cytolytic capacity. These cells are found in peripheral blood and are believed to represent a terminally differentiated effector cell that is $CD28^-$, $CD27^+$, GrB^+, $perforin^+$ and in some cases also expresses CD57. B) Differentiation of CD4 CTL in a mouse model of acute infection. In the draining lymph node, CD4 cells produce IL-2, are $CD27^+$ and $CD62L^{+/-}$. As cells differentiate and migrate to the lung, they lose surface CD62L, acquire CD43 and begin to lose CD27. The ability to secrete IFN-γ and lyse target cells is also acquired as cells migrate to the lung.

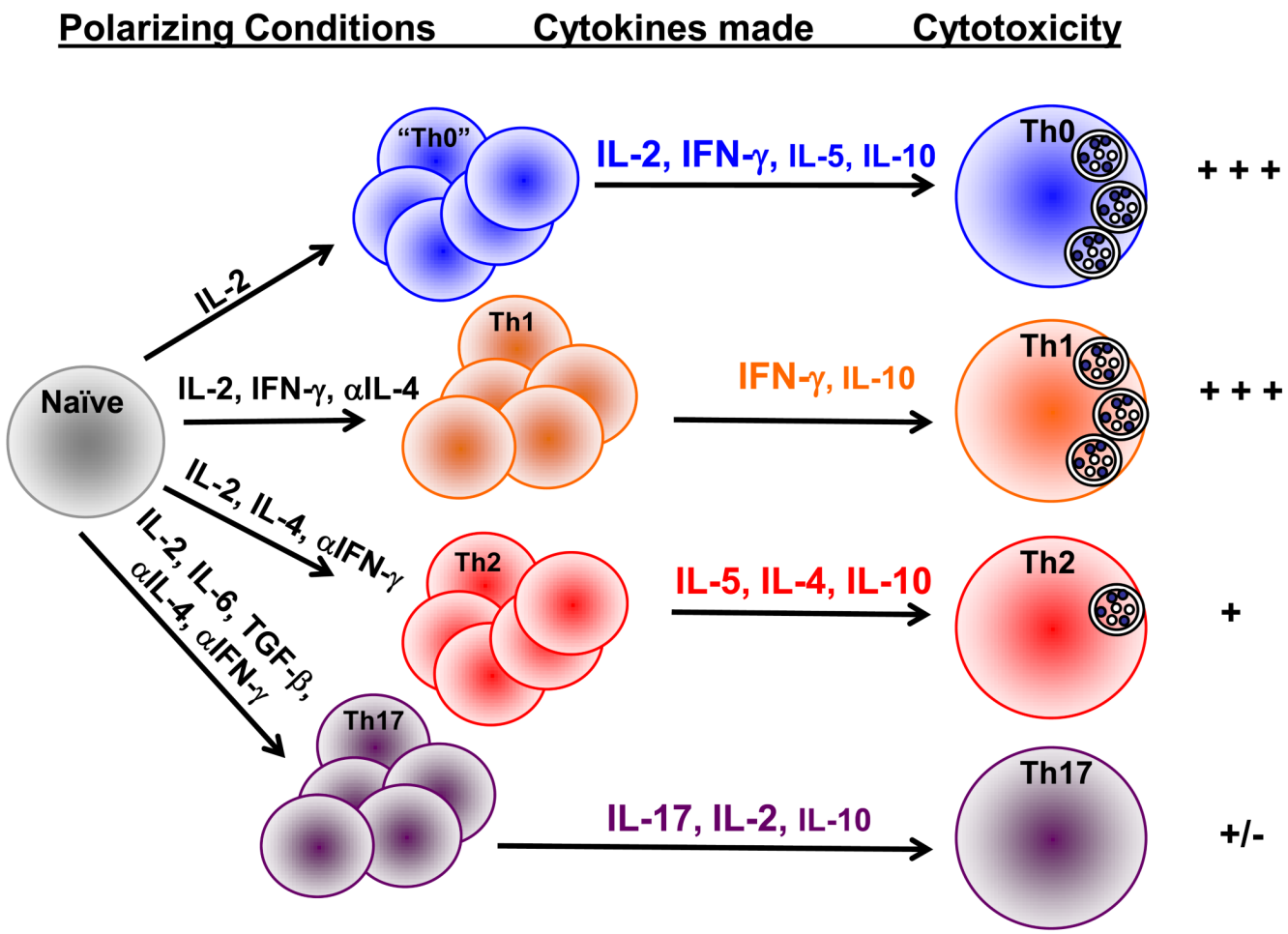

Figure 2.
Model of CD4 CTL differentiation in vitro. Naïve CD4 cells can be differentiated into various subsets based on the cytokines present early in the culture. Th0 effectors are those incubated in the presence of IL-2 and exhibit high levels of cytotoxicity. Th0 cells also secrete a combination of Th1 and Th2 type cytokines. Th1 effectors also demonstrate high levels of GrB expression and cytotoxicity while Th2 effectors are less able to lyse target cells and Th17 cells demonstrate little, if no cytolytic activity.