3 T Cell Subsets and T Cell-Mediated Immunity

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3.1 Introduction

T cell-mediated immunity is an adaptive process of developing antigen (Ag)-specific T LYMPHOCYTES to eliminate viral, bacterial, or parasitic infections or malignant cells. T cell-mediated immunity can also involve aberrant recognition of self-antigens leading to autoimmune inflammatory diseases. Ag specificity of T LYMPHOCYTES is based on recognition through the TcR of unique antigenic peptides presented by MHC molecules on antigen-presenting cells. T cell-mediated immunity is the central element of the adaptive immune system and includes a primary response by naïve T cells, effector functions by activated T cells, and persistence of Ag-specific memory T cells. T cell-mediated immunity is part of a complex and coordinated immune response that includes other effector cells such as MACROPHAGES, NATURAL KILLER CELLS, MAST CELLS, BASOPHILS, EOSINOPHILS, and NEUTROPHILS.

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3.2 Biology of the T Lymphocyte Immune Response

Each T LYMPHOCYTE expresses a unique T CELL RECEPTOR (TCR) on the surface as the result of developmental selection upon maturation in the thymus (see Chap. 2 on Hematopoiesis). Mature T LYMPHOCYTES, known as naïve T cells, circulate through blood and the lymphatic system and reside in secondary LYMPHOID ORGANS (Fig. 3.1). Naïve T cells are those that have not yet encountered foreign Ag and have not yet been activated. Antigenic peptides are presented to the naïve T LYMPHOCYTE in secondary LYMPHOID ORGANS by DENDRITIC CELLS (DC), which are the most efficient "professional" Ag-presenting cells (APC) since they also provide co-stimulatory signals for effective T cell activation. DC acquire Ag in non-lymphoid tissues throughout the body and migrate into secondary LYMPHOID ORGANS guided by inflammatory stimuli and CHEMOKINES. APC generate antigenic peptides from a pathogenic agent or a self-antigen by ANTIGEN PROCESSING and display them on the cell surface in the context of MHC molecules. The recombinant variability of individual αβTCR, on the other hand, ensures that at least a few naïve T cells will have high-affinity binding to an antigenic peptide derived from virtually any pathogen. TCR engagement triggers a cascade of intracellular signalling events resulting in activation of the naïve T cell.

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The activated T cells rapidly proliferate (clonal expansion), migrate through the tissues to the sites of Ag presence, and perform effector functions such as cell-mediated cytotoxicity and production of various CYTOKINES (soluble mediators of the immune response). Cytotoxic CD8+ T cells are very effective in direct lysis of infected or malignant cells bearing the Ag, while CD4+ T helper cells produce CYTOKINES that can be directly toxic to the target cells or can stimulate other T cell effector functions and B cell ANTIBODY production, as well as mobilize powerful inflammatory mechanisms (Fig. 3.1) (see Chap. 6 for cytokine review).

Most effector T cells will disappear after the antigenic agent is eliminated, although others will remain and form memory T cells. Unlike naïve T cells that live for a few months or effector cells that disappear at the end of the immune response, memory T cells may survive for years in LYMPHOID ORGANS and peripheral tissues. The easily activated memory T cells can perform immediate effector functions in peripheral tissues or undergo activation and clonal expansion in LYMPHOID ORGANS to mount a secondary immune response if the same Ag appears again. Memory T cells respond much faster to the Ag than naïve T cells. Thus, in the case of infection, they help to eliminate pathogens at an early stage, thereby effectively preventing the spreading of disease.

3.2.1 The Exhausted T Cell Phenotype

In the case of chronic infections or cancer, T cells may become terminally differentiated and display a phenotype which is characterized by a reduced ability to produce inflammatory cytokines and the presence of the inhibitory receptors

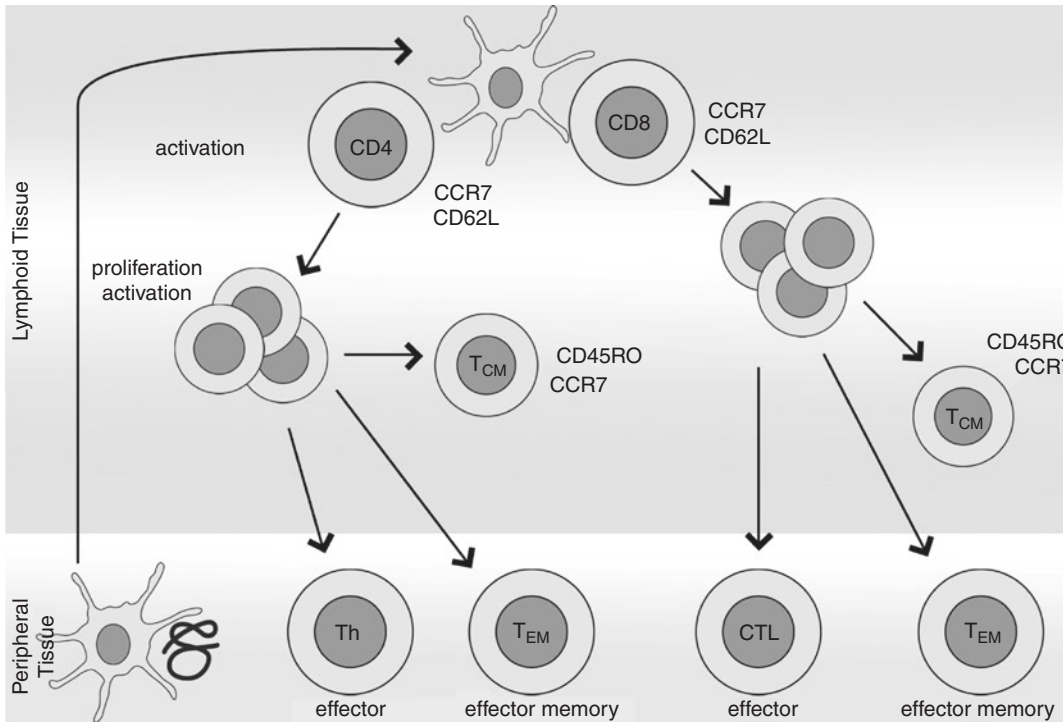

Fig. 3.1 Development of T cell-mediated responses is a sequential process. Antigen-presenting cells can take up antigen in peripheral tissues and migrate to secondary lymphoid tissues. Naïve T cells will be activated by recognition of MHC/peptide complexes on the APC, proliferate, and differentiate into effector or memory T cells. Both CD8 (CTL) and CD4 (Th) effector T cells will migrate to peripheral tissues to exert their function. In addition, memory T cells can develop into CCR7− effector memory cells (T_{EM}) that will migrate to peripheral tissues or CCD7+ central memory T cells (T_{CM}). These, in turn, can recirculate through lymphoid tissues. CCR7 is a chemokine receptor involved in T cell homing into lymphoid tissues

PD1 (programmed cell death protein 1), LAG3, TIM3, and CD160. This may be a functionally impaired or exhausted phenotype that is compatible with a progressive weakening or shutdown of the T cell response. Alternatively, it is possible that this phenotype represents a functional adaptation to a stable phenotype that balances control of immunity with avoidance of immune-mediated pathology. In this latter case, this status of the T cell would reflect "a finely tuned effector population that is optimized to fulfil a certain level of effector function and pathogen control without causing overwhelming immunopathology" [1].

3.3 Composition of the T Cell Network

3.3.1 Lymphoid Organs

The primary LYMPHOID ORGANS—the BONE MARROW and thymus—are sites of HAEMATOPOIESIS and clonal selection of T cells. The T cell-mediated immune response begins in the secondary LYMPHOID ORGANS: the spleen, lymph nodes, and organized lymphoid tissues associated with mucosal surfaces including Peyer's patches, tonsils, and bronchial, nasal, and gut-associated lymphoid tissues. The secondary LYMPHOID ORGANS have specialized T cell-rich zones where naïve T LYMPHOCYTES are concentrated; these include the PERIARTERIOLAR LYMPHOID SHEATH of spleen (PALS) and the PARACORTEX of lymph nodes. Naïve T cells reside in the spleen for just a few hours and in the lymph nodes for about 1 day before they leave via splenic veins or via efferent lymphatic vessels, respectively. Migrating naïve T cells eventually reach the bloodstream and soon after enter new LYMPHOID ORGANS, repeating the cycle until they become activated by antigenic peptides or die by neglect.

3.3.2 T Cell Subsets

Thymic selection results in the appearance of T cells with two types of TCR. The majority express Ag-binding αβ-chains in the TCR, which are disulphide-linked heterodimers of Ig superfamily proteins (Fig. 3.2) forming unique structures on each T cell. αβTCR T cells have a very diverse REPERTOIRE of Ag recognition receptors and represent mature T cells that circulate through the secondary lymph organs and develop adaptive immune responses. A small fraction of the T cells expresses γδ-chains in TCR appears to be much less heterogenic than αβTCR T cells, resides in skin and certain mucosal surfaces, and may play a role in the initial response to microbial invasion. Although the functions of γδ-TCR T cells are not fully understood, they are considered to be a relatively primitive part of the innate T cell response and will not be reviewed in this chapter.

αβTCR T cells are subdivided into several groups on the basis of lineage markers and functional activities. Two major surface co-receptor

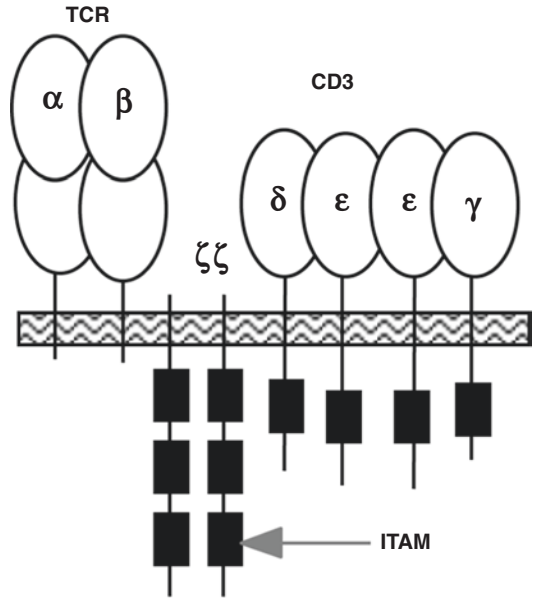

Fig. 3.2 T cell receptor complex consists of αβ-heterodimers responsible for antigen recognition and CD3 molecules involved in intracellular signalling. Immunoglobulin-like αβ-chains are formed upon gene rearrangement and have high variability among individual T cells. Non-polymorphic CD3 chains (ζ, δ, ε, γ) contain intracellular immuno-receptor tyrosine-based activation motifs (ITAMs) initiating cascades of signal transduction. ZAP70 recruitment and phosphorylation result in LAT (Linker for Activation of T cells) activation, which will induce activation of MAPK, NF-kB, and Ca signalling pathways

molecules, CD4 and CD8, define two separate T cell lineages with different functions. CD4+ cells recognize Ag in the context of MHC class II molecules (only expressed on so-called professional APC such as B cells, macrophages, and DC) and produce CYTOKINES when developed into effector T helper cells. CD8+ LYMPHOCYTES are activated by Ag peptides presented by MHC class I molecules (expressed on all nucleated cells) and form effector cytotoxic T LYMPHOCYTES (CTL).

On the other hand, the functional status of the T cells allows us to distinguish naïve, effector, and memory cells, as each of these displays extensive diversity in terms of phenotype, function, and anatomic distribution. Naïve T cells are the most homogenous representatives of CD4+ and CD8+ subsets. Upon activation, however, they can be further distinguished by their cytokine profiles. Thus, activated CD4+ T helper cells can be subdivided into Th1, Th2, Th17, and Treg subsets based on production of signature cytokines. In the case of the Th1/Th2 dichotomy, the characteristic cytokines are IFN-γ (Th1) versus IL-4 and IL-5 (Th2) [2]. CD8+ LYMPHOCYTES also can be assigned to Tc1 or Tc2 subsets according to their cytokine profile [3], although they do not produce the same quantities of CYTOKINEs as CD4+ helpers and are not efficient in B cell activation (see Chap. A3). Theoretically, both effector and memory LYMPHOCYTES of CD4+ and CD8+ lineage can be divided into subsets based on the above criteria. In addition, there are subsets of regulatory T cells that make T cell heterogeneity even more complex. Treg cells can be subdivided into naturally arising cells (nTreg) that are generated in the thymus and inducible Treg (iTreg) that are converted into Treg upon activation in the periphery [4]. Many of the specific cell surface markers representing various T cell subsets can be very useful in the design of drugs for selective manipulation of the immune response (Table 3.1).

Many proteins are upregulated or downregulated rapidly after T cell activation, like adhesion molecules or molecules involved in effector functions.

Table 3.1 Phenotypic markers associated with naïve, effector, or memory T cells

	Naïve	Effector	T_{EM}	T_{CM}
T cell subset markers				
CCR7	+++	–	+/–	+++
CD62L	+++	–	+/–	+++
CD45RO	+	+++	+++	+
CD45RA	+++	–	+	++
CD95	+/–	+++	++	+/–
Granzyme B	–	+++	+/–	–
CD25	–	+	–	–
CD127	++	+/–	+	+++
CD28	++	–	+	++

3.3.3 Naïve T Cell Markers

Naïve T cells circulating in the blood express L-selectin (CD62L), CC chemokine receptor 7 (CCR7), and leukocyte function antigen-1 (the αLβ2 integrin LFA-1). These mediate the rolling, adhesion, and extravasation of the cells through the high endothelial venules (specialized venules found in lymphoid tissues) in peripheral lymph nodes and mucosal LYMPHOID ORGANS.

Survival of naïve cells is maintained by low-affinity TCR/self-antigen interaction and signalling as well as by the presence of IL-7. These signals are normally sufficient to maintain homeostasis of naïve T cells for several months.

3.3.4 Effector T Cell Markers

High-affinity interactions of TCR with foreign Ag peptide-MHC on mature APC following activation are reflected in phenotype changes. Activated T cells express CD69 (a very early activation antigen) and CD25 (IL-2Ra). Other important surface receptors of activated T cells are CD40 ligand, which stimulates APC through binding to CD40, leading to the upregulation of CD80 (B7-1) and CD86 (B7-2) on APC, and CD28, which binds to CD80 and CD86 and propagates a co-stimulatory signal, thereby enhancing growth factor (IL-2) production and increasing T cell activation.

TNF receptor family molecules OX-40, CD27, and 4-1BB also can be found on primary

activated T cells. These receptors were found to sustain T cell proliferation and survival of activated T LYMPHOCYTES upon their binding to the corresponding ligands on the APC. At the peak of their proliferation, CD4+ effector cells were also found to change the pattern of adhesion receptors such as CD62L and sPSGL-1 (sialylated form of p-selectin glycoprotein ligand 1) and chemokine receptor CXCR5. CD8+ CTL could also be characterized by expression of perforin and granzymes, proteins required for cytolytic functions. A particular set of surface markers may predict the homing capacity of effector T cells. For example, CXCR5 receptor helps CD4+ CD62L−, sPSGL-1−, and CXCR5+ T cells to migrate into B cell-rich FOLLICLES of the lymph nodes and support ANTIBODY production. In contrast, the absence of CCR7 and CD62L on CTL allows them to migrate into inflamed nonlymphoid tissues such as the lung or gut and to clear pathogenic agents in these tissues.

3.3.5 Memory T Cell Markers

Memory T cells, unlike effector T cells, are not blasts nor do they enter the cell cycle. However, they are capable of circulating in lymphoid and nonlymphoid compartments. According to the location, memory T cells are divided into central and effector memory cells and express corresponding surface markers. For example, among three phenotypes of CD8+ memory cells that have been identified (CD45RA−, CCR7+; CD45RA−, CCR7−; CD45RA+, CCR7−), the CCR7+ T cells are non-cytotoxic central memory T cells, while CCR7− are effector memory T cells [5]. Upon contact with the appropriate Ag, effector memory cells can execute effector functions instantly, whereas central or lymphoid memory cells can rapidly proliferate, expand, and acquire effector functions. CD4+ memory T cells also appear to be heterogenic. At least two subsets of CD45RA− CD4+ memory cells have been identified in humans. The central memory cells express CCR7 and CD62L and reside in LYMPHOID ORGANS, producing IL-2 upon stimulation. Some of these have been found to migrate into certain inflammation sites depending on the expression of chemokine receptors such as CCR4, CCR6, and CXCR3. The other CCR7 subset with low CD62L expression produces IFN-γ and IL-4 upon stimulation and apparently represents effector memory cells.

3.3.6 Detection of T Cells with Peptide-MHC Multimers

It is possible to detect antigen-specific T cells with the use of peptide-MHC multimers, in most cases tetramers. This technology started with the successful detection of CD8+ T cells with avidin-biotin-based MHC I tetramers [6]. More recently it became also possible to detect CD4+ T cells with MHC II tetramers. pMHC multimers are now mostly linked to fluorochromes, which enable T cell detection through conventional flow cytometry. One of the drawbacks of tetramers for T detection is that the affinity required for pMHC binding exceeds that of T cell activation. This means that sensitivity is critical and that only TcRs with relatively high binding affinities are detected. Recent advances have shown better sensitivity with the use of higher-order multimers or by inclusion of antibodies against the pMHC multimer [7]. With this technology, monitoring of therapeutic interventions at the level of antigen-specific T cells has become a more common practice.

3.3.7 T Cells with Conserved (Invariant) T Cell Receptors

Besides peptides, T cells were shown to possess the capability to recognize antigens of a non-proteinaceous nature as well. For example, mycobacterial lipids were shown to become visible for T cells when presented in the context of a so-called nonclassical MHC molecule: CD1. Recent studies done with CD1 tetramers have shown the presence of, for example, germline-encoded mycolyl-reactive (GEM) T cell populations in the blood of TB patients. In contrast to the polymorphic classical MHC molecules, all humans do express nearly

identical CD1 proteins. In correspondence with this, the CD1-restricted T cell populations were found to bear conserved T cell receptors. Therefore, while the concept of conserved or invariant T cells initially was formed on the basis of the discovery of the CD1d-restricted NKT cells, it now appeared that besides NKT cells and the so-called mucosal-associated invariant T (MAIT) cells, additional subsets of human T cells with invariant T cell receptors, such as GEM T cells, exist [8].

3.4 Effectors of T Cell-Mediated Immunity

3.4.1 CD4+ Helpers

Two major functional T helper subpopulations are distinguished by their cytokine profiles (Fig. 3.3). Th1 cells produce mainly IFN-γ but also IL-2, TNF-α, and lymphotoxin. Th1 cells enhance pro-inflammatory cell-mediated immunity and were shown to induce delayed-type hypersensitivity (DTH) and B cell production of opsonizing ISOTYPES of IgG and mediate the response to some protozoa like *Leishmania* and *Trypanosoma*. Th2 cells secrete IL4, IL-5, IL-6, IL-10, and IL-13, promote non-inflammatory immediate immune responses, and were shown to be essential in B cell production of IgG, IgA, and IgE. Th1 and Th2 development routes appear to be mutually antagonistic. This has given rise to the model of polarization of immune response in accordance with the nature of the Ag and the surrounding CYTOKINE milieu. For example, IFN-γ and IL-12 are known to support Th1 cells, while IL-4 and IL-10 assist Th2 development. Although the evidence for the polarized cytokine secretion profiles of Th1 and Th2 is indisputable, several recent studies have shown more complex

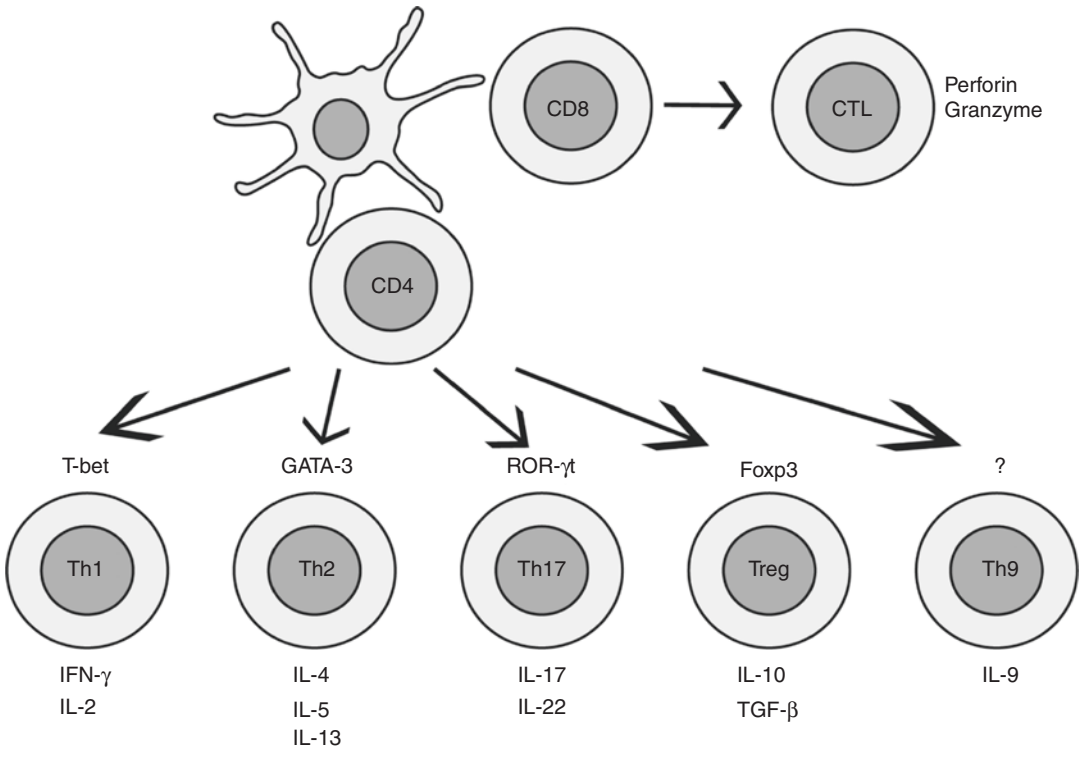

Fig. 3.3 Differentiation of effector T cells: antigen-activated T cells will differentiate into different phenotypes depending on the cytokines in the local environment and can be characterized by their cytokine profile and by transcription factors. Th1 cells produce IFN-γ and IL-2 and express T-bet. Th2 cells produce IL-4, IL-5, and IL-13 and express GATA3. Th17 cells produce IL-17 and IL-22 and express ROR γt. Treg can be divided into different subsets based on the expression of FoxP3 and/or the production of IL-10, TGF-β, and IL-35

patterns of CYTOKINE interaction in different models of immune response, including autoimmune models that are inconsistent with the simple dichotomy paradigm.

Since CD4+ T cells are central in the origin and regulation of autoimmunity, emphasis has been placed on the characterization of Th subsets and their possible roles in the inflammatory process. With the discovery that the p40 subunit of the pro-inflammatory cytokine IL-12 can not only dimerize with the p35 subunit to form IL-12 but also with p19 to create IL-23, the former dogma that IL-12-driven Th1 responses were the critical contributors to inflammation had to be revised [9]. It was found that IL-23 induced production of CD4+ T cells that secrete pro-inflammatory cytokine IL-17A. Subsequently, these cells were characterized as a separate Th subset, called Th17. Th17 cells are regarded as a major effector lineage with pro-inflammatory actions in diseases like rheumatoid arthritis, psoriasis, and Crohn's disease. Contribution of Th1 cells to inflammatory diseases is still possible, although complex, given the additional regulatory contributions of IL-12 and IFN-γ in inflammation.

Th17 cells also play a prominent role in infection. In fact, Th17 is the first subset that is generated during infection. The IL-17 receptor is expressed on fibroblasts, epithelial cells, and keratinocytes. Contact with IL-17 leads to production by the latter cell types of IL-6 and chemokines like CXCL8 and CXCL2 and GM-GSF (granulocyte-macrophage colony-stimulating factors). Altogether, this leads to recruitment of neutrophils and macrophages into the site of infection and enhances the bone marrow production of these cells. IL-22 produced by Th17 cells co-operates with IL-17 in the induction of antimicrobial peptides, such as β-defensins in epidermal keratinocytes, thereby enhancing the innate acute inflammatory response in infection.

It is anticipated that a growing spectrum of Th subset lineages will be discovered and defined by the external stimuli they respond to and the transcription factors they can induce (see Fig. 3.3). IL-12, IFN-γ, and transcription factors STAT1, STAT4, and T-bet lead to the production of Th1 cells. IL4 in combination with STAT6 and GATA-3 generates Th2 cells. Follicular T helper cells (T_{FH}) were recently defined to develop under the influence of IL-6 and transcription factor Bcl-6. Th17 cells develop in the presence of TGF-β, IL-6, and IL-23 and are characterized by the transcription factors RORγt, RORα, and STAT3. Recently, Th9 cells also were proposed, a subset that develops under the influence of IL-4 and TGF-β and that produces IL-9 [10]. Their function is associated with allergy, skin inflammatory conditions, and the control of extracellular pathogens.

There are now several subsets which may have potential to produce immunological disease. Adoptive transfer of Th1 or Th17 cells produces EAE and uveitis. Colitis in mice is produced by Th1, Th2, Th17, and Th9 cells. T_{FH} can mediate the pathogenic antibody response in experimental lupus models [11]. Th22 cells were also proposed to exist and to exert a role in inflammatory skin diseases.

3.4.2 CD8+ Cytotoxic T Lymphocytes

The CTL are derived from activated naïve CD8+ cells, proliferate in the presence of IL-2, and can expand their number many thousandfold at the peak of a primary immune response. The dramatic clonal expansion of CD8+ CTL in comparison to CD4+ cells most likely can be attributed to the relatively easy activation by the Ag/MHC class I complex and better survival in the circulation. Rapid expansion and the ability of single CD8+ CTL to destroy more than one target cell while sparing "innocent" bystanders make CTL very efficient Ag-specific effector cells. Destruction of selected cells by CTL requires the establishment of cell contact with the target cell and Ag recognition, thus initiating the release of cytolytic granules into the IMMUNOLOGICAL SYNAPSE. CTL, unlike naïve T cells, do not require co-stimulatory signals upon Ag recognition in order to kill. Therefore, they can destroy a variety of target cells bearing "foreign" Ag.

3.4.3 Mechanisms of Cell-Mediated Cytotoxicity

Two major pathways of cytotoxicity have been described in CTL: Ca^{2+}-dependent perforin/granzyme-mediated APOPTOSIS and Ca^{2+}-independent Fas ligand/Fas-mediated APOPTOSIS (Fig. 3.4). Both pathways are initiated via TCR signalling. Lytic granules (secretory lysosomes containing granzymes, perforin (PFN), and the proteoglycan serglycin (SG)) [12] appear to be transported into target cells as one complex. Granzymes are effector molecules capable of inducing APOPTOSIS in target cells via caspase-dependent and caspase-independent mechanisms. Granzymes enter into the target cell directly via plasma membrane pores formed by PFN or via receptor-mediated endocytosis. In the latter case, PFN mediates the translocation of granzymes from endocytic vesicles into the cytosol. Proteoglycan SG presumably serves as a chaperone of PFN until the complex reaches the plasma membrane of the target cells. Lytic granules represent a very efficient natural drug delivery system.

Fas-mediated APOPTOSIS is initiated by binding of Fas molecules to the target cell via Fas ligand on the CTL. The Fas molecule is a member of the TNF receptor superfamily with an intracellular "death" domain initiating caspase-dependent APOPTOSIS upon binding to Fas ligand. TCR cross-linking was shown to induce upregulation of Fas ligand expression on the cell surface of CTL and in cytolytic granules. Fas-mediated APOPTOSIS appears to be a general phenomenon not restricted to CTL. It was found to be involved in the control of cell proliferation and homeostasis among other cells.

3.4.4 Regulatory T Cells

Regulatory T cells (Treg) include more than one cell type that are critical in the maintenance of peripheral tolerance, down-modulate the amplitude of an immune response, and prevent autoimmune diseases. There is enough evidence at present to conclude that regulatory T cells participate in all cell-mediated immune responses, directly affecting Th1, Th2, Th17, CTL, and B cell reactions against "self" and "foreign" Ag. The mechanisms by which Treg exert their function are still not completely clear, but immunosuppressive cytokines such as TGF-β, IL-10, and IL-35 play an important role.

Although the majority of Treg appears within the CD4+ T cell set, suppressor activity was also reported among CD8+ T cells. In the last few years, most attention, however, was focused on CD4+ regulatory cells and particularly the nTreg

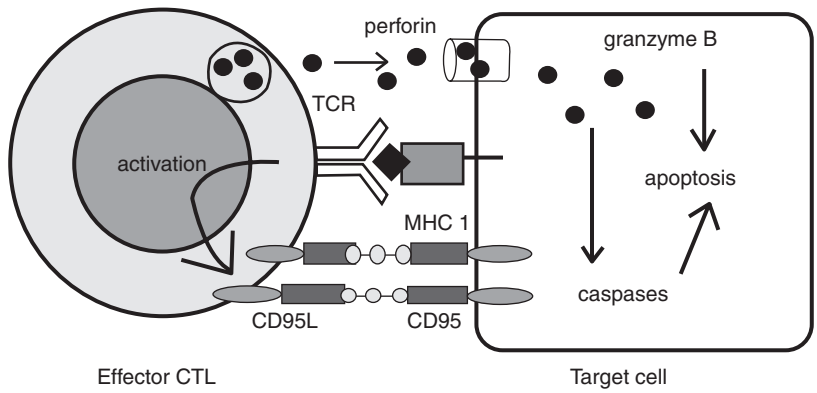

Fig. 3.4 CTL cytotoxicity can be mediated by two distinct pathways. One mechanism is via secretion of perforin and granzyme B from cytolytic granules. Perforin creates pores in the membrane of the target cell to enable granzyme B entry into the cell. Granzyme activates caspases that induce apoptosis. The second mechanism is via interaction between CD95 (Fas) and CD95L (FasL). TCR-mediated activation induces CD95L expression on the CTL. Binding of CD95 on the target cells will induce sequential caspase activation leading to apoptosis

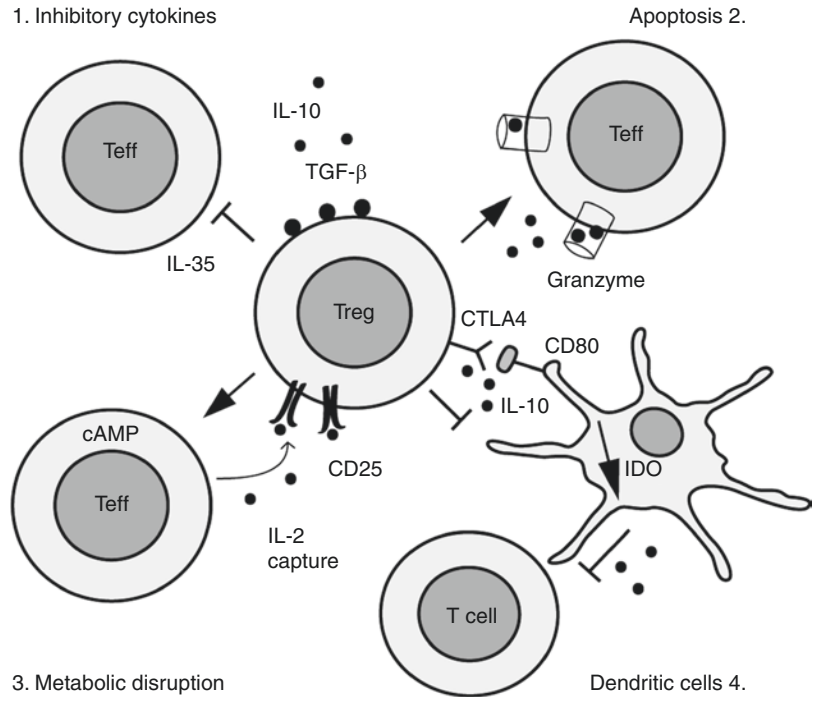

Fig. 3.5 Several mechanisms mediate Treg cell function. (1) Inhibitory cytokines such as IL-10 TGF-B and IL-35 can suppress T cell activation. (2) In some cases, cytolysis has been described as a potential suppressive mechanism, killing effector cells in a granzyme A- and B-dependent fashion. (3) Cytokine deprivation, through binding of IL-2, leads to metabolic disruption of target cells or direct cAMP-mediated inhibition. (4) DCs are targeted via direct cell-cell interactions, via CTLA4, for example, or via suppressive cytokines such as IL-10

that are characterized by constitutive expression of the α-chain of the IL-2 receptor (CD25) and the transcription factor Foxp3 [13]. nTreg arise from the thymus and represent about 10% of the total CD4 population.

Foxp3 is essential in the development and function of nTreg. The absence of functional Foxp3 results in severe systemic autoimmune diseases in mice and man. Foxp3 inhibits IL-2 transcription and induces upregulation of Treg-associated molecules, such as CD25, CTLA-4, and GITR [14], that can downregulate the immune response of adjacent cells.

In addition to nTreg, induced Treg develop in the periphery from naïve CD4+ T cells in the presence of TGF-β and IL-10 or in the absence of co-stimulation, especially in mucosal tissues. Within the population of iTreg, the heterogeneity is even more complex. Tr1 cells [15] depend on IL-10 for their induction and their suppressive action, whereas Th3 cells [16] depend on TGF-β for their suppressive action.

The inhibitory effect of all Treg primarily requires stimulation of the TCR. Upon activation, cells may mediate their function via direct cell contact through inhibitory molecules such as CTLA4, but they may also function via secretion of IL-10 and TGF-β. IL-10 can suppress differentiation of Th1 and Th2 cells directly by reducing IL-2, TNF-α, and IL-5 production but also indirectly by downregulating MHC and co-stimulatory molecules on antigen-presenting cells, thereby reducing T cell activation. The mechanism of suppression will most likely depend on the type of Treg, the nature of the immune response, the antigen, and the site of inflammation (Fig. 3.5) [17].

3.5 Mechanisms of T Cell Activation

3.5.1 Antigen Presentation

Antigenic peptides are derived by different molecular mechanisms of Ag processing, from pathogens residing either in the cytosol or in vesicular compartments of the infected cell. MHC class I molecules bind to the antigenic peptides, which originate in the cytosol of APC as a

result of a multimolecular complex of proteases (proteasomes) and are transported to the endoplasmic reticulum by TAP-1 and TAP-2 (transporter associated with Ag processing-1 and -2). The newly assembled MHC/peptide complexes in the endoplasmic reticulum are then translocated through the Golgi to the cell surface. Virtually all cells of the body express MHC class I molecules at different levels and thus present antigenic peptides to CD8+ CTL and become potential targets of destruction, depending on the Ag.

MHC class II molecules, in contrast, bind peptides deriving from pathogens that appear in intracellular vesicles of the cell or from extracellular proteins internalized by endocytosis. MHC class II molecules are transported from the Golgi to endosomes and lysosomes as a complex bound to the non-polymorphic invariant chain instead of a peptide. Subsequently, the invariant chain is degraded and replaced with peptides generated by vesicular acid proteases at acid pH in the endosomal compartments. MHC II/peptide complexes appear on the surface of only a few types of immune cells, including MACROPHAGES, B cells, and DC [18].

Another important mechanism is CROSS-PRESENTATION of Ag, a process in which "professional" APC may present an Ag transferred from other cells. This enables extracellular antigens to be presented by MHCI and to activate CTL. Several studies have shown that DC can actually initiate a T cell response against MHC class I-restricted antigens by cross-presentation. CROSS-PRESENTATION also may serve as a mechanism for T cell tolerance to self-ANTIGENS in the periphery [19].

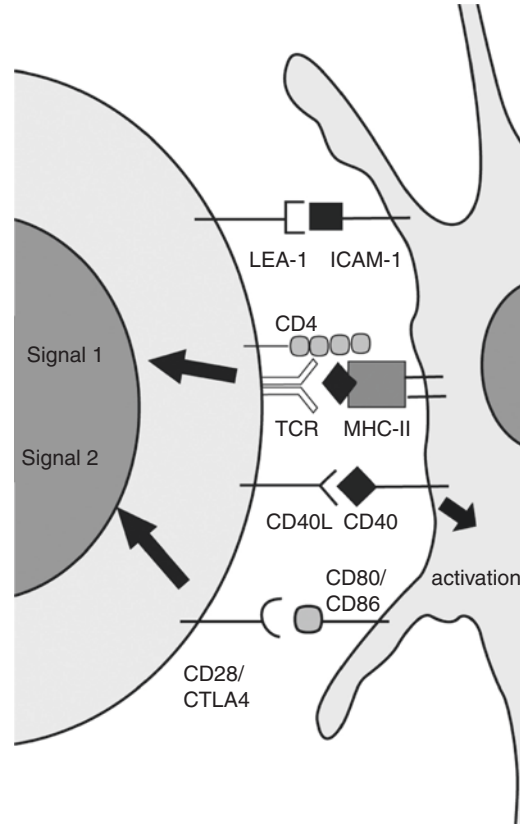

Fig. 3.6 Effective T cell activation requires interaction with multiple surface receptors on both T cells and APC. Binding of MHC class II peptide complex to the TCR and CD4 induces signal 1 in the T cell. Positive co-stimulation (signal 2) is provided by binding of CD80 or CD86 to CD28, whereas binding to CTLA4 will inhibit T cell activation. Other interactions, such as binding of LFA-1 and ICAM-1, will ensure further intensified cell-cell interactions. Binding of CD40 and CD40L will induce an activating signal in the APC, enhancing the expression of MHC molecules and co-stimulatory receptors

3.5.2 Molecular Mechanisms of T Lymphocyte Activation

Activation of naïve T cells is the most critical step in developing immunity and requires a complex interaction of TCR, co-receptors, and accessory molecules on the surface of the T cell with corresponding ligands on the APC (Fig. 3.6). TCR-Ag/MHC interaction provides an Ag recognition step and initiates intracellular signalling. Co-receptors such as CD4 and CD8 assist the TcR signal. Co-stimulatory molecules such as CD28 and CTLA-4 initiate their own intracellular signals that enhance or modulate the TCR signal. Accessory molecules such as LFA-1 or CD2 provide adhesion at the cell contact site, strengthening the interaction between the T cell and APC and allowing sustained signal transductions. The αβ-chains of TCR are non-covalently associated with invariant chains of the CD3 complex (ζ, δ, ε, and γ) (Fig. 3.2). Intracellular parts of CD3 chains include one or multiple ITAMs

(immunoreceptor tyrosine-based activation motifs). ITAMs provide sites of interaction with protein tyrosine kinases (PTK) that propagate the signalling events [20].

Src family protein tyrosine kinases *Fyn* and *Lck* phosphorylate ITAMs upon TCR cross-linking by Ag/MHC, and fully phosphorylated ITAMs recruit PTK ZAP-70 to the complex via their SH2 domains. This allows LCK to trans-phosphorylate and to activate ZAP-70. The activated ZAP-70 interacts and phosphorylates SLP-76 and LAT (Linker for Activation of T cells). SLP-76 appears to be involved in actin cytoskeleton changes, while LAT is a membrane-associated protein that upon phosphorylation provides binding sites for a number of critical signalling proteins, including Grb2, Ras, and PLC-γ. PLC-γ plays a critical role in regulation of Ca^{2+} flux as it cleaves 4,5-biphosphate (PIP2) to diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3) upon activation by PI3 kinase. DAG stimulates PKC, while accumulation of IP_3 is the initial trigger for release of intracellular Ca^{2+} that, in turn, triggers the opening of the plasma membrane Ca^{2+} release-activated Ca^{2+} (CRAC) channels. Cascade of the signalling actions eventually results in activation of transcription factors including NF-AT, ELK-1, Jun, and ATF-2 and immune gene expression.

Although the first phosphorylation events occur within a few seconds of TCR cross-linking, the sustained contact and interaction of T cells with APC is required for full T LYMPHOCYTE activation. Earlier studies of TCR engagement have focused on IMMUNOLOGICAL SYNAPSE (IS)-dynamic clustering of different surface molecules at the contact point between T cell and APC involving TCR/CD3, co-receptors, and accessory molecules [21]. Original studies reported the formation of a ring-type structure of TCR/pMHC in the centre and the formation of spatially segregated regions of supramolecular activation complexes (SMAC). These SMAC were held to initiate signal transduction. However, the current idea is that TCR signalling is initiated in microclusters of TCR, LAT, and ZAP70-mediated phosphorylation of LAT. However, for full T cell activation, prolonged TCR activation via TCR-peptide MHC interaction remains essential. IS that contains central SMAC formed on the cell surface may provide prolonged cellular interaction and sustained signalling leading to the Ca^{2+} flux, actin cytoskeleton reorganization, and full-blown T cell activation.

3.5.3 Tolerance

An essential part of T cell-mediated immunity is the development of non-responsiveness towards naturally occurring self-ANTIGENS while mounting effective immune responses against "foreign" antigens [22]. Breakdown of self-tolerance will result in the development of autoimmune diseases. Self-reactive T cells, both CD4+ and CD8+, have been shown to be responsible for initiating and mediating tissue damage in many experimental animal models of organ-specific autoimmunity as well as in human studies.

Immunological tolerance is achieved by different mechanisms at different stages. Initially, potential self-reactive T LYMPHOCYTES are deleted during T cell development in the thymus. High-affinity interaction of TCR on immature thymocytes with self-Ag on thymic stromal cells results in apoptosis and elimination of such T cells in the process known as negative selection. T cells with TCR of low to moderate affinity to self-ANTIGENS escape from the thymus and migrate to the periphery. These T cells are normally "ignorant" to self-Ags or develop tolerance after initial activation.

Although the Ag-specific TCRs of T cells do not possess an intrinsic mechanism to distinguish self from non-self peptides, the activation by self-Ag is different to that by "foreign" Ag, mainly due to the absence of co-stimulatory signals from non-activated APC. This is in contrast to activated APC that upregulate co-stimulatory molecules during inflammation, infections, or other pathological conditions. Partial activation of T cells in the absence of co-stimulatory signals leads, instead of activation, to the state of T cell unresponsiveness towards further stimulation, also known as ANERGY [23].

In most cases, co-stimulatory molecules will direct T cell response towards either activation or tolerance. Simple absence of co-stimulatory signals was shown to induce ANERGY in effector T cells in vivo and in vitro, while naïve T cells may require a negative signal of CTLA-4 engagement to develop anergy and become tolerant.

Self-reactive cycling T cells may also undergo programmed cell death after re-exposure to the same Ag in the process called activation-induced cell death (AICD). AICD is mediated by death receptors (FAS/FAS ligand interaction of CD4+ T cells and by TNFRII/TNF interaction of CD8+ T cells) that involve interaction of caspase-dependent, death-inducing signalling complexes (DISC).

Peripheral tolerance can be also controlled by immune cytokine divergence and by regulatory T cells. Both natural and adaptive CD4+ regulatory cells have been implicated in the regulation of the autoimmune response. Thymus-derived CD25+ nTreg cells suppress other types of cell activation by largely unknown mechanisms. They require strong co-stimulatory signals for induction and maintenance, with Foxp3 expression. Adaptive (antigen-induced) regulatory T cells are generated in the periphery by suboptimal antigenic signals and rely on CYTOKINES such as IL-10 and TGF-β for suppression. These cells of varying phenotype appear often under special conditions such as chronic viral infections. Regulatory T cells present new possibilities for the treatment of autoimmune disorders and for the graft survival of transplanted organs.

3.6 Summary

T cell-mediated immunity includes priming of naïve T cells, effector functions of activated T helpers and CTL, and long-term persistence of memory T cells. Development of an effective immune response requires proper activation of T LYMPHOCYTES by APC in secondary LYMPHOID ORGANS and migration of the responding T cells to the sites of Ag presence in the body. The efficiency of T cell activation in LYMPHOID ORGANS depends on the concentration of an antigenic peptide and affinity of TCR towards the Ag/MHC complex and is facilitated by inflammatory stimuli, co-stimulatory signals, and CYTOKINES. CD8+ naïve T cells develop into effector CTL after interaction with APC, while CD4+ naïve T cells differentiate into T helper cells of major T helper types: Th1 (producing IL-2, IFN-γ, TNF-α, and LT-α) or Th2 (IL-4, IL-5, IL-6, IL-10, and IL-13).

Absence of inflammatory stimuli may induce insufficient activation of DENDRITIC CELLS resulting in induction of ANERGY and apoptosis among T cells instead of activation and productive response. This may serve as a mechanism of tolerance to self-Ags. Circulation and extravasation of T LYMPHOCYTES are orchestrated by multiple adhesion receptors whose expression and avidity are modulated by CYTOKINES and chemokines. In the process of mediating effector functions, some activated T cells undergo activation-induced cell death (AICD), while others undergo activated T cell autonomous death after the inflammation wanes, thus terminating the immune response. Only a small population of Ag-specific memory cells remains in LYMPHOID ORGANS and throughout the tissues for a long time after the immune response is over. When exposed to the Ag a second time, memory cells rapidly acquire and mediate effector functions, thereby preventing spread of pathogenic infection.

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