T Helper 1 Cells Overview

What are Th1 cells?

As their name suggests, T helper (Th) cells provide helper functions to other cells of the immune system—especially the antigen-presenting cells (APCs) such as **macrophages**, **dendritic cells**, and B cells—and are important for their activation and maturation. There are distinct subsets of $CD4^+$ Th cells, including Th1, **Th2**, **Th17**, and **T regulatory cells**, each activated by a specific set of cytokines and transcription factors and characterized by the cytokines they secrete and effector functions they perform.

Th1 cells derive from the alpha:beta lineage of T cells and recognize antigens presented by major histocompatibility complex (MHC) class I or II molecules. Th1 cells play important roles in the identification and eradication of intracellular pathogens such as viruses and bacteria, including *Mycobacterium tuberculosis*, *Mycobacterium leprae*, and Leishmania. These pathogens typically reside in phagocytic vesicles within cells such as macrophages and often evade intracellular killing by preventing lysosomal fusion. Th1 cells help to activate macrophages against these pathogens and overcome these microbial evasion strategies. In contrast, Th2 cells, which are the other major subset of $CD4^+$ T cells, help to recognize extracellular pathogens such as helminths and parasites and activate B cell–mediated antibody responses.

On this page:

Development of Th1 cells

Thymic phase

The first phase of T cell development takes place in the thymus (**Figure 1**), where T cell commitment is driven by Notch1 signaling, along with the expression of specific transcription factors such as TCF1, GATA3, and Bcl11b. All T cell subsets of the alpha:beta lineage can trace their development to a common T cell progenitor (Thp) that proceeds through a double-negative (DN) ($CD8^-$ $CD4^-$) phase, followed by a double-positive (DP) phase ($CD8^+$ $CD4^+$) before the thymocytes become single-positive (SP) $CD8^+$ or $CD4^+$ thymocytes.

MHC molecules play important roles in the maturation of SP cells. $CD4^+$ T cells recognize antigenic peptides that are presented by MHC class II molecules mediated by the CD4 co-receptor binding to invariant regions in the MHC class II chain. The importance of MHC–co-receptor interaction in thymocyte development is underscored by studies in transgenic mice expressing either MHC class I or class II genes. Absence of MHC class II genes results in the development of only $CD8^+$ cells, and absence of MHC class I genes results in the development of only $CD4^+$ cells, thus destroying the CD4/CD8 balance in T cell populations.

DP cells have an inherently short life span of 3–4 days, unless they are rescued by the rearrangements of their TCR genes. This rescue of DP thymocytes from apoptosis and their subsequent maturation into SP cells is known as positive selection, during which T cells recognizing self MHC complexes are selected for further development. A converse process, termed negative selection, occurs when T cells strongly recognizing a self peptide:self MHC complex in the thymus is destroyed by apoptosis, thus preventing harmful activation of self-reacting mature T cells [**1**].

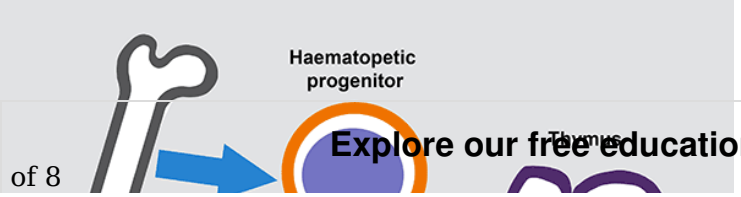
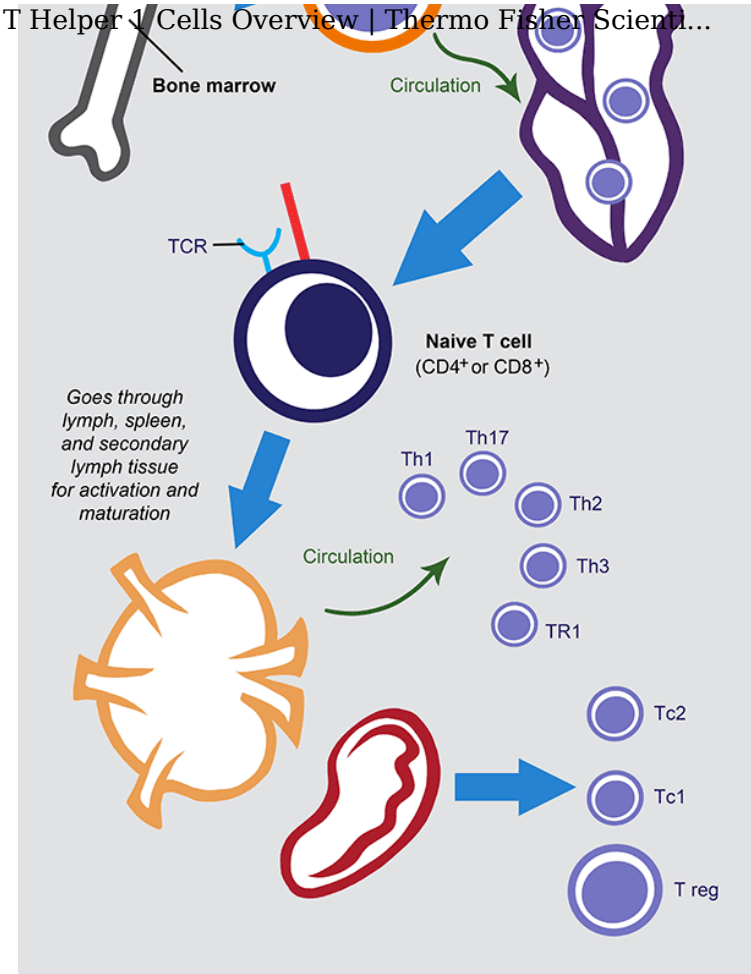


Figure 1. Development of T cells.



Differentiation into Th1 effector cells

At the end of the thymic phase, uncommitted naive CD4+ T (Th0) cells leave for the secondary lymphoid organs to scout for antigen presented by MHC class II molecules. Upon antigen recognition, the naive T cells undergo clonal expansion and differentiation into the different effector subtypes, while a few develop into memory T cells. The differentiation lineages are heavily influenced by a milieu of cytokines, transcription factors, co-stimulatory signals, and adhesion molecules.

The two cytokines that play a critical role in Th1 differentiation are IFNγ and IL-12. IL-12 secreted by APCs upon T cell engagement drives differentiation into Th1 effector cells through the activation of STAT4 transcription factor. STAT4 induces IFNγ production, which is another driver towards Th1 differentiation by activating transcription factors STAT1 and Tbet. Tbet is considered the master regulator of Th1 differentiation since it induces more expression of IFNγ, leading to a positive feedback loop to strengthen the Th1 response [**1,2,3**].

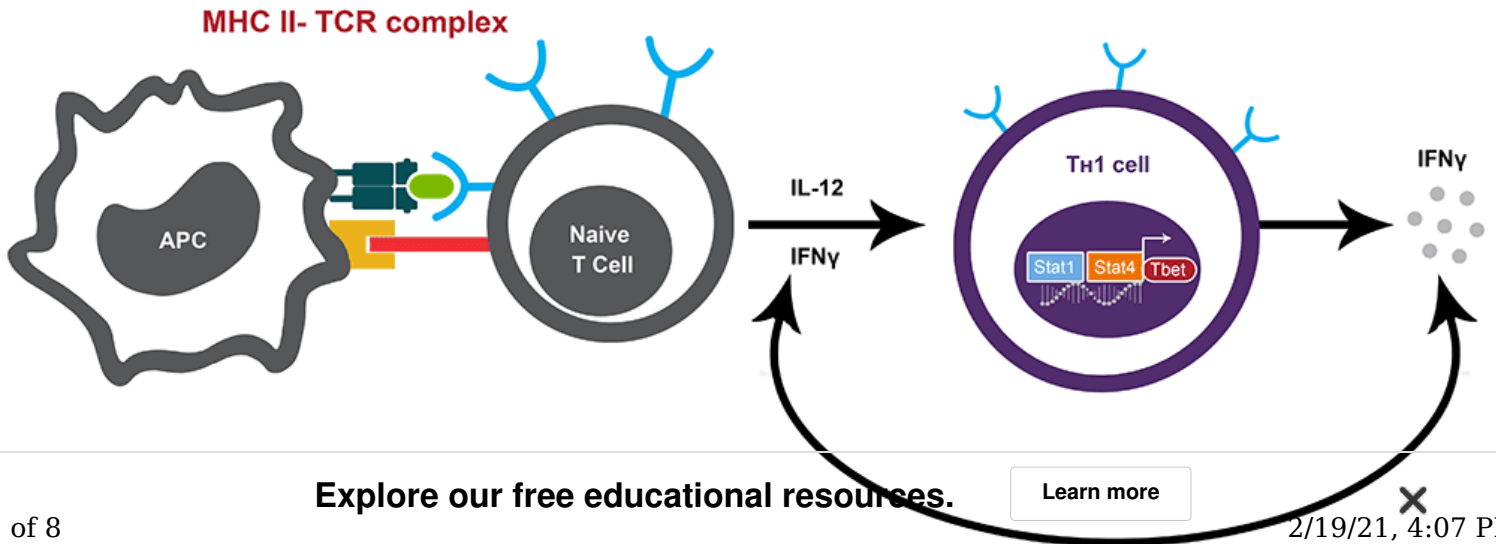


Figure 2. Th1 development and IFNγ mediated positive feedback loop to aid Th1 expansion.

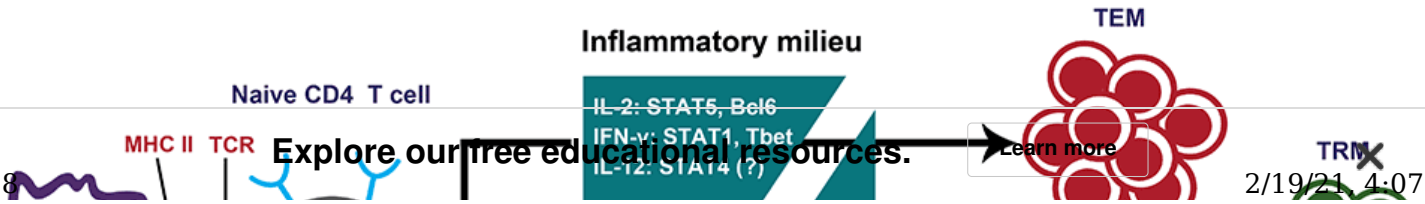
Table 1. Gene expression and cytokine changes that drive Th1 cell development*.

	Naive CD4 T cell	Th1 effector T cell
Cytokines that drive development	IL2	IL-12 IFNγ
Characteristic transcription factors and signaling proteins	Lck ZAP-70 LKLF	Tbet STAT1 STAT4
Cell surface markers	CD3 CD28 CD4 CD62L CD45RA CD5 CCR7	CD3 CD28 CD4 CD45RO CD44 Fas FasL (type 1) IL-12 R2 CD119 (IFNγR1) CD183 (CXCR3) CD186 (CXCR6) CD191 (CCR1) CD195 (CCR5) CD212 (IL-12Rβ1) CD218a (IL-18Rα) (in mouse, not human) CD254 (RANKL) CD366 (TIM3)
Cytokines that are secreted		IFNγ TNFα GM-CSF IL3 LTα LTβ CXCR3 CCL2 IL2 IL10

*Adapted from Murphy KM, Weaver C (2017). Janeway's Immunobiology (9th edition). New York: Garland Science.

Memory CD4+ T cells

Antigen recognition also gives rise to memory Th1 cells, which express the characteristic marker CD45RO. Memory cells are important for a robust and faster immune reaction following subsequent antigenic stimulation. There are three kinds of $CD4^+$ memory cells: central memory T cells (TCM), effector memory T cells (TEM), and tissue-resident memory T cells (TRM). TCM cells have a high proliferative potential, whereas TEM possess low proliferative potential but high inflammatory effector functions. There are different models for the differentiation of CD4+ memory T cells (reviewed in [**4**]). In one model, it is hypothesized that Th1 effector cells could give rise to TEM memory cells, although there is some plasticity in memory T cell differentiation. The subset of memory T cell differentiation is guided by the cytokine environment and transcription factor activity.



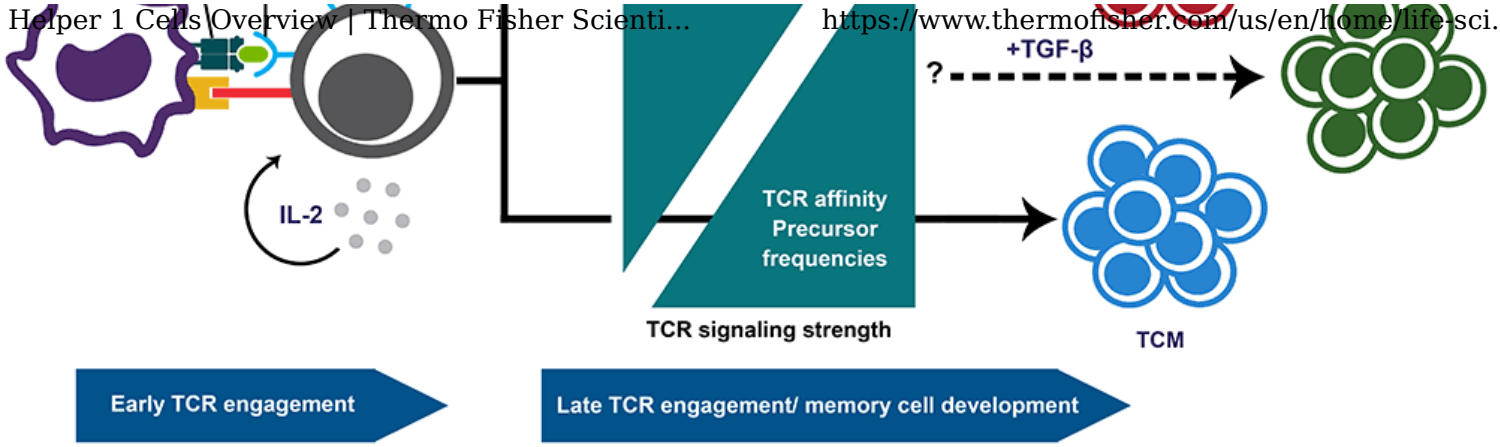


Figure 3. Factors influencing memory CD4 T cell differentiation. *Reproduced with permission from [5] under the Creative Commons Attribution 4.0 International License (**http://creativecommons.org/licenses/by/4.0/**).*

Co-stimulatory molecules and their role in Th1 differentiation

Co-stimulatory (or co-inhibitory) molecules are expressed on APCs and interact with molecules on T cells to enhance or decrease the signal generated. In addition, they can also modulate the signal to cause changes in transcription programs being initiated. Therefore, these molecules have a significant role to play in T cell differentiation and in the fates of the different lineages [**2,3,5**].

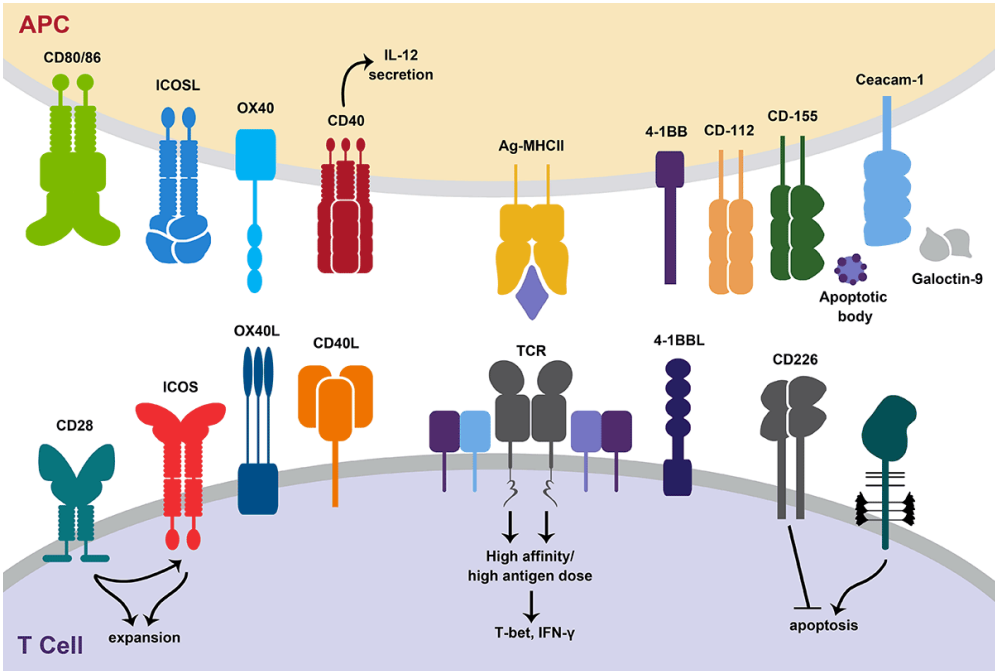


Figure 4. Co-stimulatory molecules and their role in Th1 differentiation. Adapted from [4].

Table 2. Co-stimulatory molecules important for Th1 differentiation and function.

Name of molecule	Stimulation or Inhibition	Interaction partners on APCs	Notes
CD28	Co-stimulatory/ co-inhibitory	B7	**Co-stimulatory:** high antigenic dose or high-affinity TCR binding favors Th1 lineage. **Co-inhibitory:** prolonged CD28 engagement favors Th2 lineage.
ICOS	Co-stimulatory/co-inhibitory	ICOSL	Can be co-stimulatory or inhibitory for different infections. For example, co-stimulatory for Salmonella infections, but inhibitory for Listeria infections.
CD226 (DNAM-1)	Co-stimulatory	CD155(PVR), CD112 (Nectin-2)	Involved in Th1 activation and expansion. Upregulated

Tim3	Co-inhibitory	Galectin 9	Contributes to Th1/Th2 imbalance in allergic asthma. Inhibition of Tim3 increases Th1 cytokines.

Effector functions of Th1 cells

The main effector functions of Th1 cells are in cell-mediated immunity and inflammation, including the activation of cytolytic and other effector functions of other immune cells such as macrophages, B cells, and $CD8^+$ cytotoxic T lymphocytes (CTLs). One of the first steps in Th1-mediated activation of other immune cells is the interaction of CD40 Ligand (CD40L) on the surface of Th1 cells with CD40 expressed on the surface of macrophages, B cells, and dendritic cells. Effector Th1 cells also secrete copious amounts of IFNγ that, besides further expanding the Th1 population, also activates cytolytic activities of macrophages through the induction of more than 200 target genes. This increased cytotoxic activity in macrophages is key to killing intracellular pathogens such as viruses, intracellular bacteria, and protozoa. A classic example of Th1-dependent immune response is during Mycobacteria infection. Mycobacteria escape lysosomal fusion within macrophages; however, peptides derived from these pathogens displayed by MHC Class II on the surface of infected macrophages lead to activation of Th1 responses, which turns on the cytolytic properties of macrophages.

In addition to clearing intracellular infection, Th1 responses play crucial roles in activating $CD8^+$ cytotoxic T lymphocytes to target and destroy tumors, in addition to leading to increasing CTL survival and memory. CD40 also activates class switching in B cells to produce IgG2a antibodies [**5**].

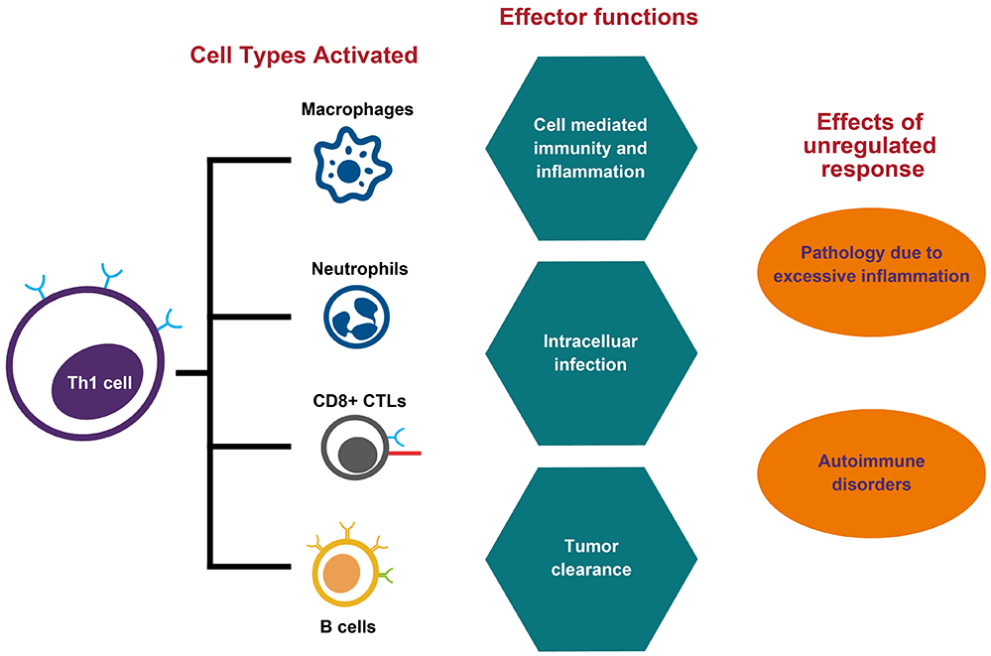


Figure 5. Th1 effector functions.

Table 3. Cytokines secreted by Th1 cells and their functions.

Cytokines and markers expressed by Th1 cells	Functions
IFNγ	Stimulates expansion of Th1 cells; activates cytolytic activities of infected macrophages and dendritic cells
TNF alpha	Acts on endothelium, along with LTα and causes macrophages to enter the site of infection from the local blood vessels
GM CSF	Together with IL3 provides distal functions to activate bone marrow–derived macrophage differentiation
IL3	Together with GM CSF provides distal functions to activate bone marrow–derived macrophage differentiation
CD40L	Binds CD40 that is expressed on macrophages, B cells, and dendritic cells to activate effector functions on these cells. Involved in antibody class switching in B cells to produce more IgG2a, which by activating the complement pathway promotes opsonization.

FASL	Activates death receptor signaling in FAS containing targets
LTα, LTβ	Activates neutrophil killing to eliminate pathogens; also has a role in inflammation
CXCR3	Facilitates migration of Th1 and CD8 T cells to peripheral sites of infection and inflammation; also enables interaction of T cells with APCs and the amplification of Th1 loop
CCL2	Acts as a chemoattractant for macrophages to accumulate at the site of infection
IL2	Activates $CD8^+$ CTLs for expansion and cytolytic activities
IL10	Regulates Th1 activation

Tools to study Th1 cells

Isolation of Th1 cells

Obtaining a Th1 population can be achieved by two different ways:

- Isolating a T cell fraction (for example, naive $CD4^+$ T cells) by magnetic bead–based separation or FACS, and then subjecting these cells to *in vitro* activation and differentiation into specific Th1 sub populations.
- Isolating the Th1 subpopulation directly from peripheral blood mononuclear cells from whole blood.

T cell fraction isolation usually starts with a Ficoll* density gradient centrifugation to separate out the buffy coat, which is the top interphase layer containing T and B cells and monocytes (PBMCs). From the PBMCs, the T cell population can be isolated by either positive or negative selection. In positive selection, T cell–specific antibodies are used to enrich for the T cell population; in negative selection, antibodies for other populations such as B cells are used to specifically remove these populations from the sample. In a positive selection, T cells or T cell subsets are first labeled with biotinylated antibodies directed toward T cell lineage markers such as CD3 or CD4, followed by the addition of **Invitrogen Dynabeads streptavidin-coated magnetic beads**. A detailed explanation and description for Ficoll density gradient separation and a scheme for magnetic bead–based positive selection for T cells can be found in the **T Cell Stimulation and Proliferation eLearning Course**.

Alternately, FACS can be used to isolate naive $CD4^+$ T cells from a sample. For flow cytometry analysis, a combination of fluorescent markers for cell surface proteins and intracellular proteins (such as cytokines and transcription factors) is typically used.

Once a $CD4^+$ population has been isolated, T cell activation and stimulation can be achieved by crosslinking and stimulation of the TCR and CD28 receptors using anti-CD3 and anti-CD28 antibodies. Magnetic beads covalently coupled to anti-CD3 and anti-CD28 antibodies are commercially available. For example, Invitrogen Dynabeads magnetic beads induce optimal levels of T cell activation and expansion by inducing both co-stimulatory signals through T cell expressed CD28 and primary signaling via the T cell receptor. *In vitro* differentiation protocols for Th1 are available for treating naive $CD4^+$ T cells with IL-12 and IFNγ [**5**]. We recommend reading Flaherty S, Reynolds JM (2015) **Mouse naive $CD4^+$ T cell isolation and *in vitro* differentiation into T cell subsets** for markers and a gating scheme to isolate naive $CD4^+$ T cells.

* Ficoll is a registered trademark owned by GE Healthcare companies

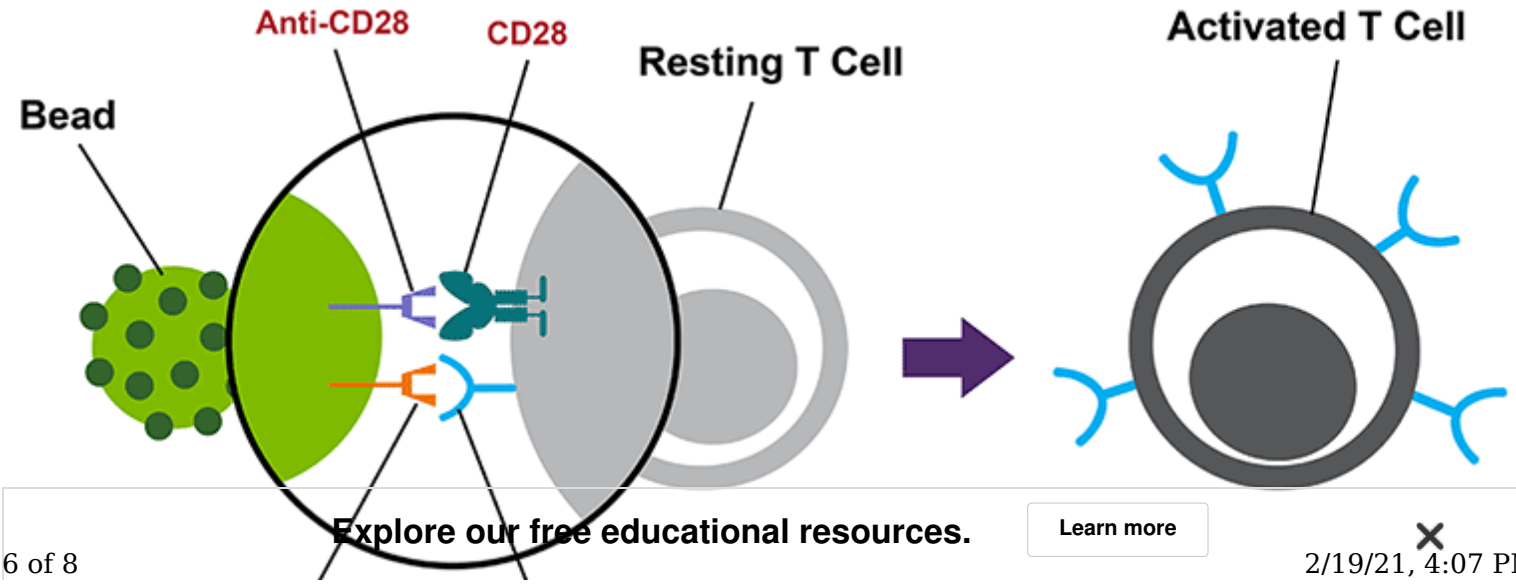


Figure 6. ***In vitro*** **activation of T cells using antibodies against CD3 and CD28.**

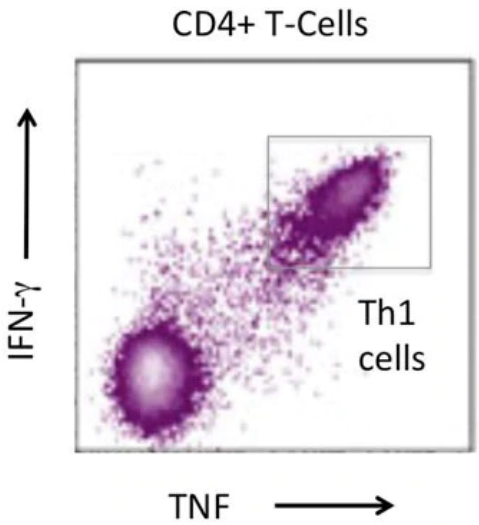


Figure 7. Identification of Th1 subtype by flow cytometry using antibodies for characteristic cytokines.

Tools for profiling and identification of Th1 cells

Cytokine profiling is commonly used to classify the Th cell subtype and also to quantify the amount of cytokines secreted. Cytokine ELISAs can be used to monitor T cell dependent cytokine secretion in response to activation and lineage-specific differentiation at the population level. **Cytokine ELISA kits** suitable for the detection and quantitation of hundreds of individual cytokines are commercially available. These kits are typically sold as 96-well plates pre-coated with the capture antibody, and contain the detection antibody, as well as standards, buffers, and accessory reagents. Assay sensitivities are commonly in the picogram range.

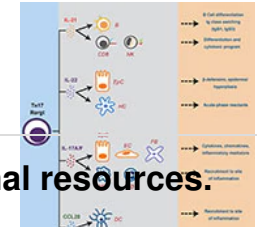
While ELISAs can be used to measure the secretion of individual cytokines, advances in **Luminex multiplexing technology** allow for the high-throughput detection of multiple cytokines in a single sample or reaction well. The simultaneous measurement of multiple cytokines is achieved using a bank of antibodies bound to microspheres dyed with fluorophores of differing intensities. Quantitation is accomplished using a sandwich assay approach in combination with a Luminex detection system. The **Invitrogen Th1/Th2 Cytokine 11-Plex Human ProcartaPlex Panel** detects a panel of 11 cytokines using the Luminex platform.

In addition to ELISA-based methods, another common and powerful tool to study Th1 and other immune populations is flow cytometry. Whereas an ELISA measures the amount of cytokines secreted, flow cytometry can be used to profile cells based on both surface-expressed or intracellular markers, as well as cytokine expression. In addition, flow cytometry can be used to quantify the Th1 population with respect to other populations. Optimized Multicolor Immunofluroscence Panels (OMIPs) published in the journal *Cytometry Part A* (Wiley Online Library) describe the use of a combination of specific antibodies and fluorophores for the extensive characterization of cell types by flow cytometry. **OMIP-030** (Characterization of human T cell subsets via surface Markers) and **OMIP-008** (Measurement of Th1 and Th2 cytokine polyfunctionality of human T cells) are useful OMIPs to study Th1 populations. Flow cytometry–based identity and testing of Th1 cells can also be performed using the **Invitrogen eBioscience Essential Human Th1/Th17 Phenotyping Panel with Staining & Stimulation Reagents**.

▸ **References**

Related articles and resources

T-helper cell paradigm poster
Discover how Naive Th cells play a central role in modulating the immune response.



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Immune Cell Guide
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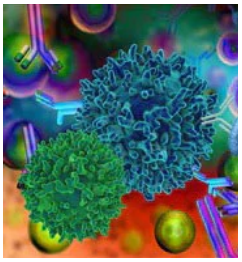

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