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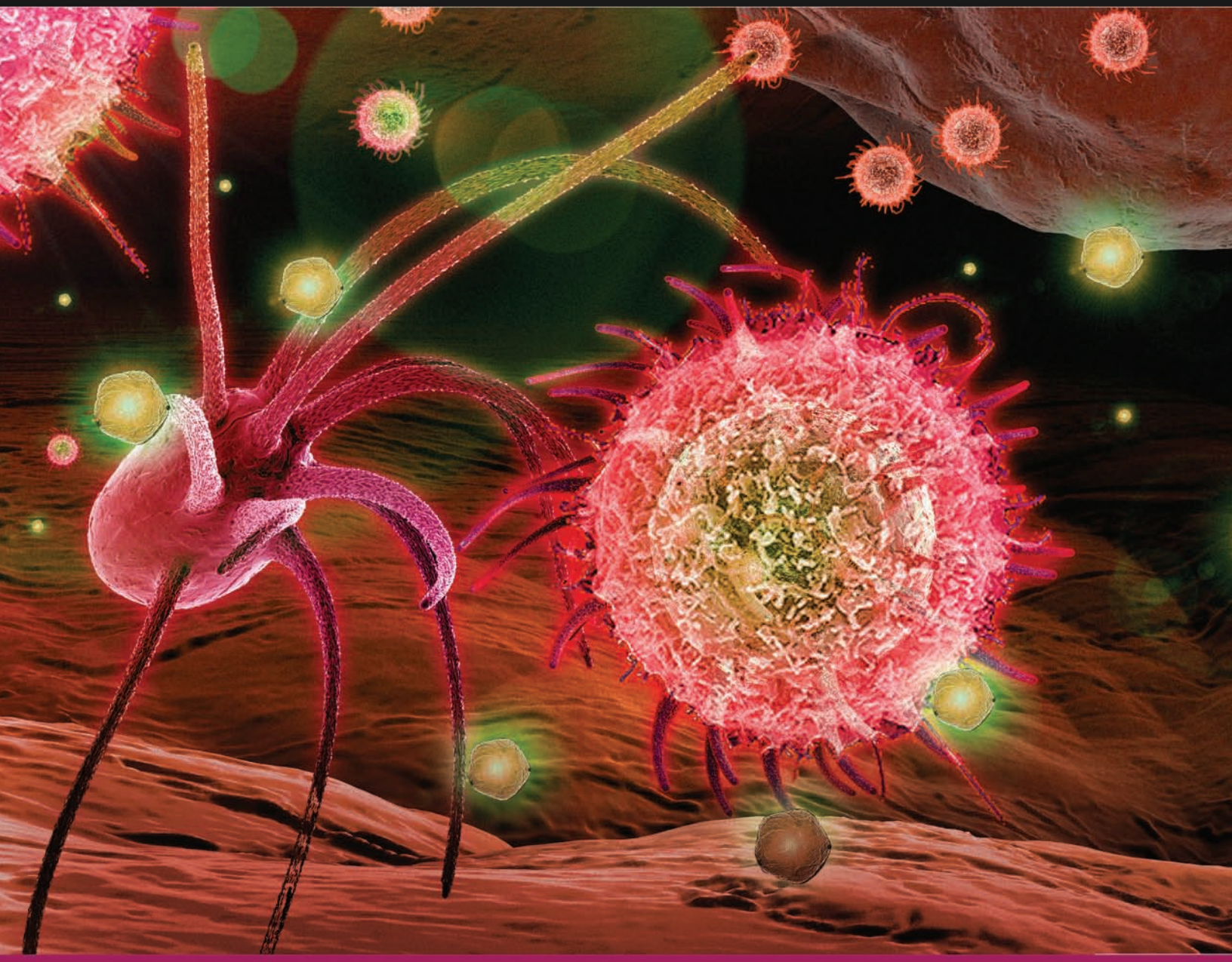

The Path to Polarization: Th_1 and Th_2 Cells

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Recent Developments in Th_1 and Th_2 Development: The Role of Cytokines and Notch Ligands

Cytokines are protein mediators that play a critical role in shaping the magnitude and type of leukocyte responses following antigen or pathogen exposure. Deviations in the levels or repertoire of cytokines produced can have significant ramifications in immune regulation and susceptibility/progression in autoimmunity, cancer, and infectious diseases. On the basis of cytokines that they produce, antigen-stimulated $CD4^+$ T lymphocytes can be divided into subsets. Prior to stimulation, naïve T cells are considered precursors (designated Th_P). Upon antigen stimulation, $CD4^+$ Th_P become Th_0 cells and produce a variety of cytokines. Depending on the environmental conditions (cytokine milieu and antigen presenting cells, for example), Th_0 cells can become polarized into either T helper 1 (Th_1) or T helper 2 (Th_2) cells. Th_1 and Th_2 cells secrete a defined, and largely non-overlapping, subset of cytokines acting on distinct target cell populations.

The target cell populations and cytokines secreted by Th_0, Th_1, and Th_2 lymphocytes are shown in Table I. Defining Th_1 and Th_2 lymphocytes by cytokine production requires that multiple cytokines be analyzed simultaneously. The investigator can accomplish this through multi-color flow cytometric staining, traditional ELISAs, or multiplexed ELISAs, such as the Q-plex™ array (please see page 8 for experimental data using the Q-plex™ Th_1/Th_2 array).

Experimental studies designed to understand the development and function of differentiated $CD4^+$ cytokine-producing subsets has been a predominant theme in immunology for over 15 years. Generally speaking, Th_1 cells direct cell-mediated inflammatory reactions to effectively control intracellular pathogens including viruses and some bacteria, principally through the production of IL-2 and IFN-γ, with TNF-α and IL-15 also playing a role. IL-15, originally reported to be produced by macrophages, dendritic cells, and stromal cells only, was later shown to be constitutively produced by T cells and required for homeostatic T cell proliferation.[1, 2] In lymphoid organs, IL-15 is expressed in the T cell zone where Th_1 cytokine production is most pronounced.[3] Interestingly, IL-15 levels are considerably higher than IL-2 (40× greater than IL-2) in lymphoid organs where active immune responses are occurring, although IL-15 is produced with kinetics similar to that of IL-2. Taken together, these data imply that IL-15 may also play a key role in the generation and maintenance of a Th_1 response.

In contrast, Th_2 cells direct and enhance B cell activation and antibody production (particularly IgE) to promote allergic reactions and eosinophilic inflammation through the production of cytokines that include IL-4, IL-5, IL-6, IL-10 and IL-13.[4] Although Th_1 cells have been shown to be "cross-inhibitory" for the reciprocal Th_2 phenotype (and vice versa), these functionally distinct cytokine-producing cells do not appear to be permanently polarized. Indeed, a large body of evidence suggests that there is conversion between Th_1 and Th_2 populations under some activation conditions, allowing flexibility of the immune response responding to various pathogens.[5] In cases where the immune response is strongly fixed towards one phenotype, the prevalent cytokine imbalance has been associated with disease pathogenesis. For example, a dominant Th_2 response has been associated with atopic dermatitis, asthma, and the outgrowth of a number of cancers,[6–10] while a dominant Th_1 response has been described for sarcoidosis, tuberculosis, and collagen-induced arthritis.[11–13]

Table I

Cytokine Production and Cellular Targets of Th_0, Th_1, and Th_2 Cells

$CD4^+$ Th Designation	Cytokines Produced	Cellular Targets
*Th_0	IL-2, IL-3, IL-4, IL-5, IL-6, IL-13, IFN-γ, TNF-α, TNF-β, GM-CSF	
Th_1	IL-2, IL-3, IL-15, IFN-γ, TNF-α, TNF-β, GM-CSF	B cells, macrophages, NK cells, cell mediated immunity
Th_2	IL-4, IL-5, IL-6, IL-13, IL-21, TNF-β, GM-CSF	B cells, mast cells, eosinophils

* Naïve Th cells become Th_0 cells with characteristics of both Th_1 and Th_2 cells. Further stimulation induces deviation of Th_0 cells towards either a Th_1 or Th_2 cell with a distinct set of cytokines and cellular targets.

Recent Developments in T Helper Subset Development

Differentiation of $CD4^+$ T cells into Th_1 and Th_2 populations was thought to be driven largely by cytokines. Numerous experimental models documented that IL-12 production by activated macrophages and IFN-γ production by NK cells promotes naïve T cell differentiation into Th_1 cells while inhibiting Th_2 cell differentiation. On the other hand, IL-4 was shown to effectively promote Th_2 differentiation and inhibit Th_1 cell differentiation. CD4 T cell differentiation is ultimately controlled by antigen presenting cells that give the T cells information about the invading pathogens. Dendritic cells recognizing DNA, RNA, or bacterial structures such as lipopolysaccharide (LPS) have been shown to promote Th_1 differentiation while recognition of fungal products, toxins such as cholera, or parasitic nematodes have been shown to lead to strong Th_2 T cell responses.[14]

How might dendritic cells direct the development of T helper subsets? Dendritic cells can direct development of Th_1 cells through the production of cytokines such as IL-12[15] and IL-18.[16, 17] IL-18 was originally described as a cytokine that induced IFN-γ in an IL-12-dependent manner.[17] Recent studies using IL-12 -/- mice have shown that stimulation of IL-12 -/- T cells with IL-18 and anti-CD3 failed to induce IFN-γ production, but markedly upregulated IL-12Rβ2 expression.[16] The current model for the role of IL-18 in Th_1 development suggests that while IL-18 cannot drive Th_1 cells alone, it is an important cytokine to enhance IL-12 signaling and promote Th_1 development especially when low IL-12 levels are present. Although IL-12 appears to be a key player in Th_1 development, Th_1 responses have been shown to occur in IL12 -/- mice,[18] implying that other mechanisms and pathways exist. Further, while IL-4 is an important differentiation factor for Th_2 cells,[19] IL-4 production by non-T cells is not absolutely required for Th_2 cell differentiation.[20] Recently, other cytokines such as IL-33[21] and the Th_2-associated cytokine IL-21[22] have been shown to potently induce Th_2 cytokine production and suppress IFN-γ production to downregulate Th_1 responses. Investigations aimed at identifying alternate mechanisms driving Th_1/Th_2 development have also led to the discovery that in addition to the cytokine milieu, Notch ligands can direct differentiation of $CD4^+$ T cells into Th_1 and Th_2 lineages. The cytokine- and Notch-driven Th_1 and Th_2 pathways illustrated in Figure 1 show the importance of both the antigen presenting cell and cytokine environment in the differentiation of polarized Th_1 and Th_2 populations.

Notch and Notch Ligands

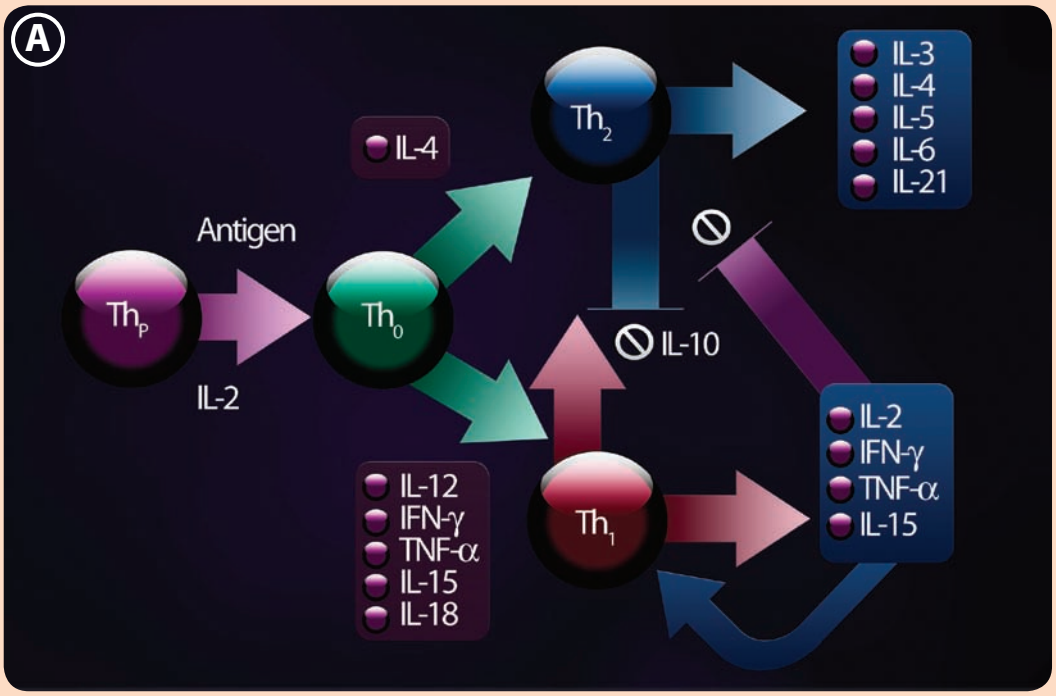
Mammals express four Notch genes and at least five genes for Notch ligands.[23] Notch proteins are single-pass transmembrane receptors containing an epidermal growth factor domain and a Notch/LIN-12 repeat important for ligand binding and ligand-independent signaling, respectively. The intracellular domain of the Notch proteins contains a RAM domain, ankyrin repeats, nuclear localization sequences, a homopolymer repeat of glutamine and a proline-glutamate-serine-threonine rich domain.[23] The five Notch ligand genes fall into two conserved families, namely Jagged (Jagged1 and Jagged2) and Delta-like (Delta1, Delta3, Delta4). When Notch ligands stimulate the Notch receptors, γ-secretase cleaves the intracellular region of Notch resulting in the translocation of this domain into the nucleus.[23–24] In the nucleus, the Notch intracellular domain (Notch-IC) binds to RBP-Jκ (also known as CBF-1 and CSL) converting it from a transcriptional repressor to a transcriptional activator. Several target genes are known to be regulated by Notch signaling (for example, Hes1, Hes5, and pTα), although it is not fully understood how these genes contribute to the biology of Notch/Notch ligand or the potential regulation of Th_1 and Th_2 cells.[25–26]

Notch and Notch Ligands in Th_1 and Th_2 Development

Both Notch1 and Notch2 are expressed in naïve T cells and expression can be increased by T cell receptor crosslinking.[27] Notch ligands, on the other hand, are found on antigen presenting cells including splenic $CD11c^+$ cells (Delta4, Delta1, Jagged2) and activated B cells (Jagged1). Pathogen-derived products are known to upregulate specific Notch ligands on dendritic cells,[28] suggesting that the type of infectious agent and activation stimuli may control expression of these ligands on antigen presenting cells. Stimulation of naïve $CD4^+$ T cells with the Delta1 ligand has been shown to promote Th_1 differentiation that is at least partially independent of IL-12.[29] Supporting studies have shown that Delta1 ligand can increase T-bet expression[29] and that Delta1-expressing fibroblasts can increase IFN-γ production by activated T cells.[28] On the other hand, stimulation with Jagged1 ligand has been shown to increase IL-4 production[28] and induce antigen-specific T regulatory cells.[30–32]

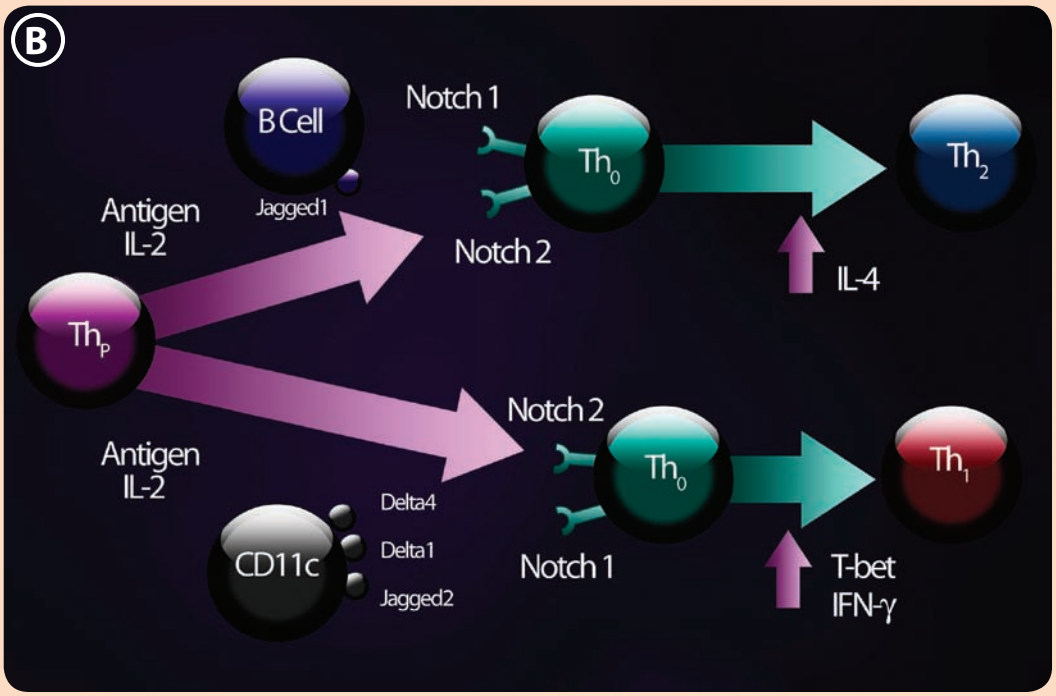
Recently, investigators have engineered mice that conditionally express a dominant negative protein capable of blocking

Figure 1



Recent evidence suggests that there are at least two pathways that can direct development of Th_1 and Th_2 cells.

A. In the classical pathway, cytokines present in the environmental milieu can influence the development of a Th_0 cell that has encountered antigen and IL-2 towards either a Th_1 or Th_2 pathway. Distinct cytokines are produced depending on the type of antigen and antigen presenting cell involved in the immune response. In cases where high levels of IL-4 are present, Th_0 cells can be directed to develop into Th_2 cells that produce IL-3, IL-4, IL-5, IL-6, and IL-10. In cases where IL-2, IL-12, IL-18, IFN-γ, and TNF-α predominate, Th_0 cells can be directed to develop into Th_1 cells producing high levels of IL-2, IFN-γ, and TNF-α. Th_1 and Th_2 cells can be cross-regulated by reciprocal cytokines. IL-2 and IFN-γ can inhibit Th_2 cytokine production; while high levels of IL-10 can inhibit Th_1 cell development. Th_1 and Th_2 cells are not fixed and conversion between these cellular phenotypes can occur depending on cytokines and other micro-environmental signals.



B. More recently, Notch ligands present on antigen presenting cells have been shown to influence Th_1 and Th_2 cell development. A Th_0 cell that has encountered antigen and IL-2 can express cell surface Notch 1 and Notch 2. When these T cells encounter antigen presenting cells expressing distinct Notch ligands, they can be directed to develop into either Th_1 or Th_2 cells. B cells expressing the Notch ligand Jagged1 increase IL-4 production in the environment and drive the development of Th_2 cells. On the other hand, $CD11c^+$ cells expressing the Notch ligands Delta1, Delta4, and Jagged2 increase levels of the transcription factor T-bet and enhance IFN-γ production resulting in the development of Th_1 cells.

Table II

Th_1 Related Markers

Name	Description/Function	References
IL-2	Cytokine produced by activated Th_1 cells driving cell-mediated inflammatory reactions and Th_1 proliferation	47
IFN-γ	Production by NK cells promotes naïve T cell differentiation into Th_1 cells by increasing IL-2 receptor beta 2 expression	48
IL-12	Production by activated macrophages promotes naïve T cell differentiation into Th_1 cells and enhances IFN-γ production	49, Reviewed in 50
IL-15	Production by epithelial/stromal cells, muscle cells, and T cells shown to inhibit T cell apoptosis and promote homeostatic proliferation through autocrine/juxtracrine loops	2
IL-18	Production by macrophages and monocytes shown to upregulate IFN-γ production and IL-12Rβ2 expression in T cells. IL-18 proposed to be a growth and differentiation factor for Th_1 cells	16, 17
IL-23	Production by dendritic cells enhances IFN-γ production to promote Th_1 differentiation	Reviewed in 50
IL-27	Synergizes with IL-12 to drive IFN-γ production, induces T-bet expression to promote Th_1 differentiation	51
Delta1 ligand	Notch ligand shown to promote Th_1 development partially independent of IL-12. Can increase IFN-γ production by activated T cells	28, 29
CXCR3 (CD183)	Receptor for IP-10, Mig, and I-Tac. Preferentially expressed in Th_1 cell populations	37
CCR5 (CD195)	Receptor for RANTES, MIP-1α, β. Preferentially expressed on Th_1 cell populations	37
CD226	DNAM-1, associated with LFA-1, expressed preferentially on the surface of Th_1 cell populations	46
Tim-3	T cell Ig domain, mucin domain-3 protein preferentially expressed on the surface of T-bet$^+$, Th_1 cells	42, 43, 44
STAT-4	Transcription factor that regulates IL-12 receptor beta 2 expression and drives Th_1 development in an IL-12 receptor beta2 independent manner	52
T-bet	Transcription factor overexpressed in Th_1 cell populations, may control GATA-3 levels to promote Th_1 development	35
Chandra	Four pass transmembrane protein expressed preferentially on Th_1 cell populations	39

Notch-IC signaling though the RBP-Jκ/CSL transcriptional activation pathway.[33] This dominant negative protein can effectively block signaling from all four Notch receptors. Using infectious disease models, it was shown that in the absence of Notch signaling in $CD4^+$ T cells failed to mount protective Th_2 responses against the gastrointestinal helminth *Trichuris muris* while they exhibited normal, protective Th_1 responses required to control *Leishmania major* infections.[33] These findings are in direct contrast to previous studies using pharmacological inhibitors of Notch signaling where Th_1 cell differentiation was shown to be inhibited.[34]Clearly, additional studies will be required to fully elucidate the roles of the various Notch proteins and ligands in the control of Th_1 and Th_2 cell differentiation and function.

Recent Developments in Th_1 and Th_2 cell Identification

Although Th_1 and Th_2 cells have been largely characterized by the cytokines they produce (Table I), a large body of work

Table II *continued*

Th_2 Related Markers

Name	Description/Function	References
IL-4	Cytokine driving development and proliferation of Th_2 cells and inhibiting Th_1 cells. Stimulates antibody production by B cells	47, 53
IL-5	Th_2 cytokine stimulating antibody production by B cells and bone marrow progenitor production of eosinophils	59
IL-10	Th_2 cytokine that inhibits IL-2 and IFN-γ production. Inhibits IL-12 production by dendritic cells to inhibit Th_1 cell development	60, 61
IL-13	Th_2 cytokine that promotes B cell synthesis of IgE	62
IL-21	Production by activated T cells shown to stimulate B cell proliferation after CD40 cross-linking. IL-21 is differentially expressed in Th_2 populations	22
IL-33	Reported to drive the expression of Th_2 related cytokines including IL-4, IL-5, and IL-13. In vivo administration of IL-33 leads to severe pathological changes in mucosal organs as a result of strong Th_2 immune response	21
Jagged1	Notch ligand shown to promote Th_2 development. Can increase IL-4 production by activated T cells	29
CCR3	Chemokine receptor for MIP-1α, MIP-1β, RANTES, MCP-2, 3, and 4, and eotaxin 1, 2, and 3. Preferentially expressed on Th_2 cells	36, 37
CCR4	Chemokine receptor for MIP-1α, RANTES, MCP-1. Preferentially expressed on Th_2 cells	36, 37
CD81	Expression on T cells can enhance Th_2 differentiation	64, 65
CXCR4 (CD184)	Receptor for CXCL12, co-receptor for HIV-1. Preferentially expressed on Th_2 cells	66
CCR8 (CDw198)	Receptor for CCL1. Preferentially expressed on Th_2 cells	67, 68
ICOS (CD228)	Differentiated Th_2 cells express more ICOS than differentiated Th_1 cells. ICOS stimulation is predominantly active in Th_2 dominated immune responses	38, 69
Tim-1	T cell Ig domain, mucin domain-1 protein expressed at higher levels on the surface of Th_2 cells	44
Tim-2	T cell Ig domain, mucin domain-2 protein preferentially expressed on the surface of Th_2 cells	45
T1/ST2	IL-1R family member preferentially expressed on the surface of Th_2 cells; crosslinking can increase Th_2 proliferation and cytokine production	40, 41
STAT6	Transcription factor shown to regulate Th_2 recruitment and effector function as well as eosinophilia	54, 58
GATA-3	Transcription factor upregulated in developing Th_2 cells, enhances IL-4, IL-5, and IL-13 production. Downregulates IFN-γ production	55, 56, 57

suggests that there are a number of cell surface and intracellular markers that are differentially expressed on Th_1 and Th_2 populations. Examples of these markers are listed in Table II. Several of these markers merit additional discussion.

Th_1 and Th_2 cell development is known to be controlled by key transcription factors. A large body of evidence suggests that signal transducer and activator of transcription 4 (STAT4) regulated IL-12 receptor beta 2 expression and T-bet expression are associated with the development of Th_1 cells while GATA-3, STAT6, and c-Maf are associated with the development of Th_2 responses. Recent experimental data suggests that naïve T cells tend toward a Th_2-like development through GATA-3 (which down regulates the STAT4/IL-12 receptor beta 2 pathway) unless T-bet is activated.[35] Once T-bet is activated, GATA-3 is downregulated suggesting that the role of T-bet is to control GATA-3 levels rather than to positively regulate the IFN-γ gene as originally proposed.[35]

There are only a few cell surface markers that can reliably

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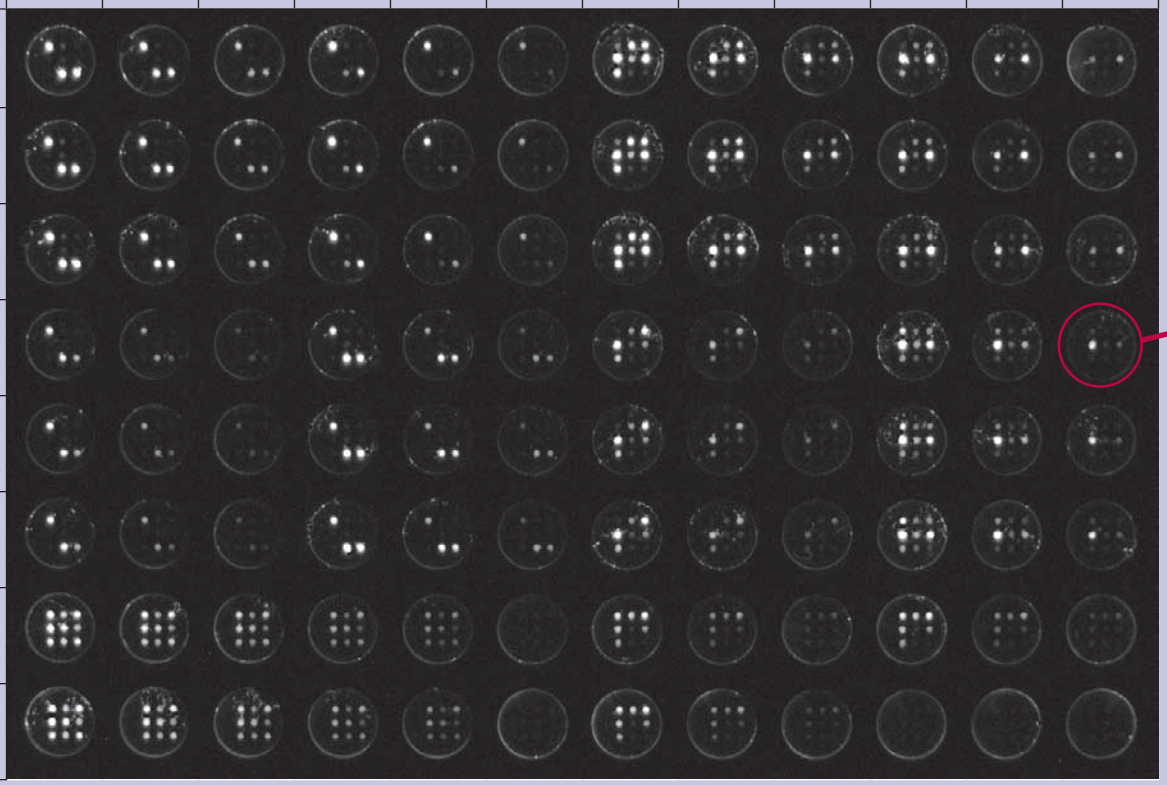
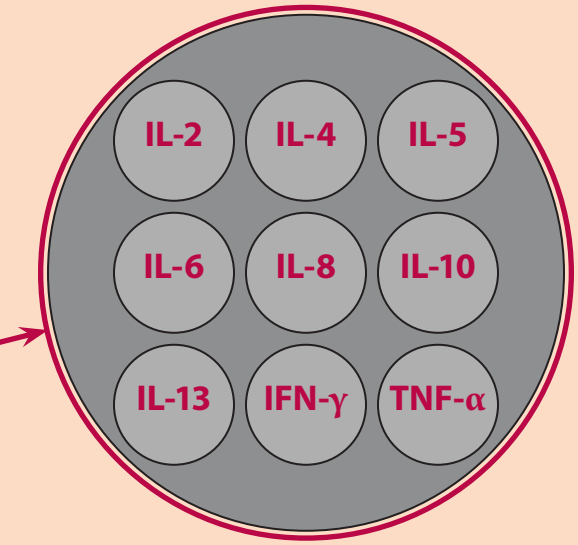


	1	2	3	4	5	6	7	8	9	10	11	12
A	**Unk. #1** Neat	**Unk. #1** 1:5 dil.	**Unk. #1** 1:25 dil.	**Unk. #3** Neat	**Unk. #3** 1:5 dil.	**Unk. #3** 1:25 dil.	**Unk. #5** Neat	**Unk. #5** 1:5 dil.	**Unk. #5** 1:25 dil.	**Unk. #7** Neat	**Unk. #7** 1:5 dil.	**Unk. #7** 1:25 dil.
B	**Unk. #1** Neat	**Unk. #1** 1:5 dil.	**Unk. #1** 1:25 dil.	**Unk. #3** Neat	**Unk. #3** 1:5 dil.	**Unk. #3** 1:25 dil.	**Unk. #5** Neat	**Unk. #5** 1:5 dil.	**Unk. #5** 1:25 dil.	**Unk. #7** Neat	**Unk. #7** 1:5 dil.	**Unk. #7** 1:25 dil.
C	**Unk. #1** Neat	**Unk. #1** 1:5 dil.	**Unk. #1** 1:25 dil.	**Unk. #3** Neat	**Unk. #3** 1:5 dil.	**Unk. #3** 1:25 dil.	**Unk. #5** Neat	**Unk. #5** 1:5 dil.	**Unk. #5** 1:25 dil.	**Unk. #7** Neat	**Unk. #7** 1:5 dil.	**Unk. #7** 1:25 dil.
D	**Unk. #2** Neat	**Unk. #2** 1:5 dil.	**Unk. #2** 1:25 dil.	**Unk. #4** Neat	**Unk. #4** 1:5 dil.	**Unk. #4** 1:25 dil.	**Unk. #6** Neat	**Unk. #6** 1:5 dil.	**Unk. #6** 1:25 dil.	**Unk. #8** Neat	**Unk. #8** 1:5 dil.	**Unk. #8** 1:25 dil.
E	**Unk. #2** Neat	**Unk. #2** 1:5 dil.	**Unk. #2** 1:25 dil.	**Unk. #4** Neat	**Unk. #4** 1:5 dil.	**Unk. #4** 1:25 dil.	**Unk. #6** Neat	**Unk. #6** 1:5 dil.	**Unk. #6** 1:25 dil.	**Unk. #8** Neat	**Unk. #8** 1:5 dil.	**Unk. #8** 1:25 dil.
F	**Unk. #2** Neat	**Unk. #2** 1:5 dil.	**Unk. #2** 1:25 dil.	**Unk. #4** Neat	**Unk. #4** 1:5 dil.	**Unk. #4** 1:25 dil.	**Unk. #6** Neat	**Unk. #6** 1:5 dil.	**Unk. #6** 1:25 dil.	**Unk. #8** Neat	**Unk. #8** 1:5 dil.	**Unk. #8** 1:25 dil.
G	**Std.** Neat	**Std.** 1:2 dil.	**Std.** 1:4 dil.	**Std.** 1:8 dil.	**Std.** 1:16 dil.	**BL**	**Unk. #9** Neat	**Unk. #9** 1:5 dil.	**Unk. #9** 1:25 dil.	**Unk. #9** Neat	**Unk. #9** 1:5 dil.	**Unk. #6** 1:25 dil.
H	**Std.** Neat	**Std.** 1:2 dil.	**Std.** 1:4 dil.	**Std.** 1:8 dil.	**Std.** 1:16 dil.	**BL**	**Unk. #9** Neat	**Unk. #9** 1:5 dil.	**Unk. 96** 1:25 dil.	**BL**	**BL**	**BL**

Unk=Unknown samples Std=Antigen Standards BL=Blank dil = dilution



Human Th_1/Th_2 Cytokine Array

Figure 2 The Q-plex™ human cytokine Th_1/Th_2 array (Cat. No. 599101) was used to analyze unknown samples for Th_1 and Th_2 associated cytokines. The chemiluminescent image of the plate captured by CCD camera is shown along with a template with the location of the samples, antigen standards, and blanks. An individual well template shows the location of the various cytokine capture antibodies. After sample or pooled antigen standards are added to the spotted capture antibodies, biotinylated secondary antibodies are added, followed by HRP-conjugated streptavidin. The addition of chemiluminescent substrate allows the visualization of captured cytokine that can be quantitated by comparing pixel intensities to that obtained with the antigen standard curves for each individual cytokine. Examples of antigen standard curves for individual cytokines from the plate imaged are shown in Figure 3 on page 10. For the nine unknown samples tested, the quantitative results (shown for a single sample dilution) can be grouped into Th_1 responses, Th_2 responses, or mixed responses as summarized in Table III and shown in selected bar graphs (Figure 4 on page 11). For the samples that showed mixed responses, one of these showed increased production of IL-5 characteristic of allergic or parasitic responses, whereas the other showed an increase in IL-6 and IL-10 (which has been associated with various viral infections such as herpesvirus 8). For more information on the Q-plex™ assay including an instructional video, technical manuals, and FAQs, please visit www.biolegend.com.

Table III

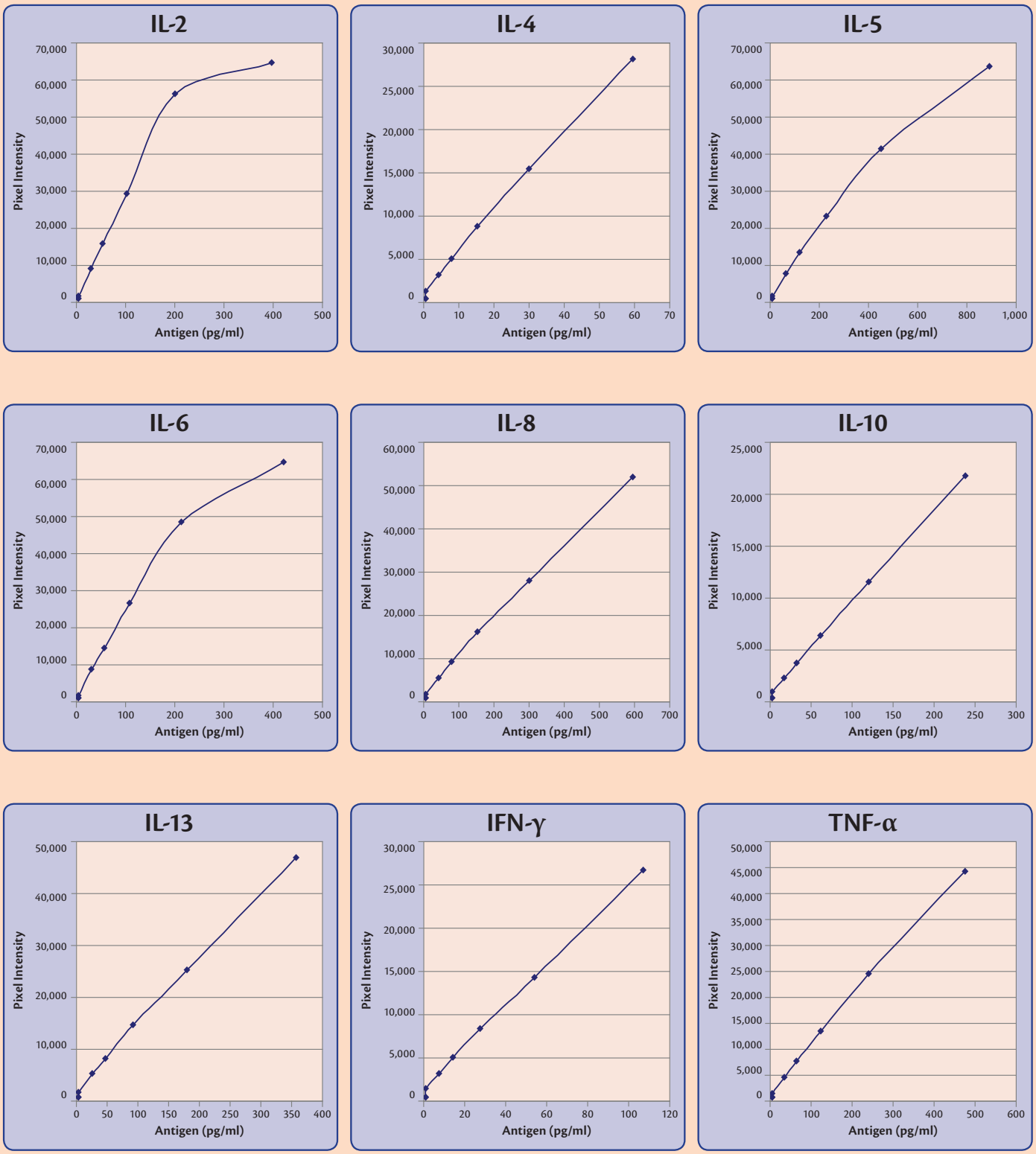
Antigen Concentrations in Unknown Samples for Nine Individual Cytokines.

Unknown Sample	IL-2 (pg/ml)	IL-4 (pg/ml)	IL-5 (pg/ml)	IL-6 (pg/ml)	IL-8 (pg/ml)	IL-10 (pg/ml)	IL-13 (pg/ml)	IFN-γ (pg/ml)	TNF-α (pg/ml)	
#1	400	0.8	ND	4.4	1.9	0.6	0.4	270	726	Th_1 Profiles
#2	41	ND	ND	ND	0.4	ND	ND	35	31	
#3	396	0.4	ND	0.8	ND	0.1	ND	6.2	103	
#4	48	0.1	ND	ND	1.2	7.4	ND	226	367	
#5	8.8	41	405	425	100	731	199	3.2	8.8	Th_2 Profiles
#6	0.1	3.5	169	86	14	21	32	ND	0.6	
#7	0.9	3.1	22.7	163	11.2	731	20.7	ND	3.3	
#8	25.7	2.2	17.5	425	40	180	14	2.1	2.7	Mixed Responses
#9	23.2	9.6	104	16	1.6	19.6	14.5	ND	0.3	

ND=Not detectable

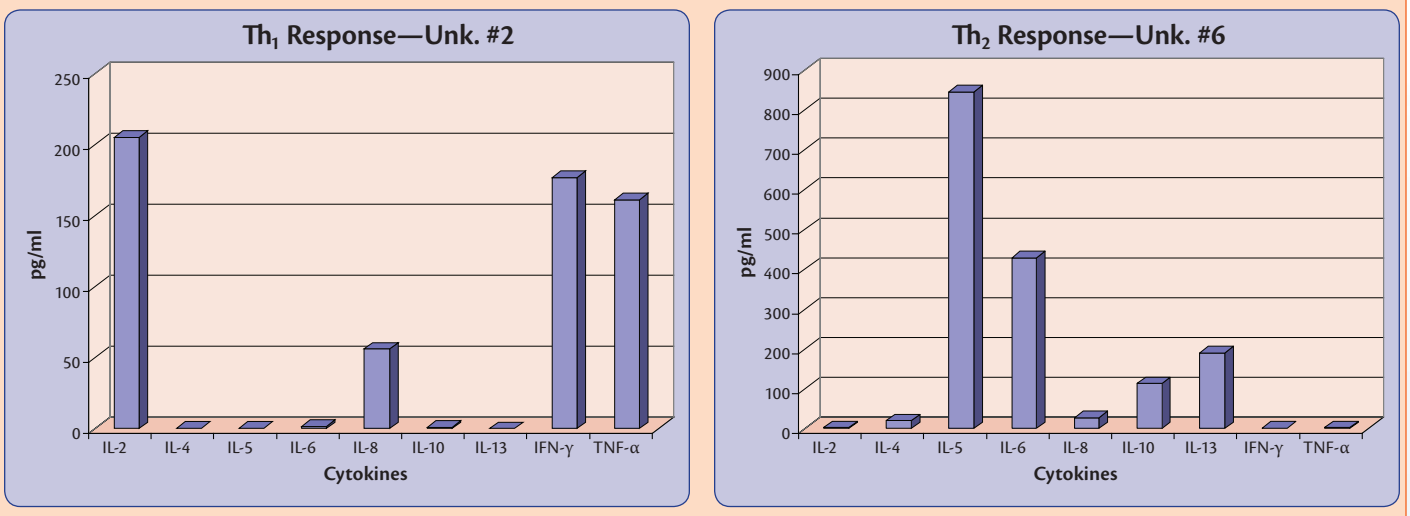
Cytokine values were calculated based on the standard curves obtained with the pooled antigen standards obtained from the plate image shown in Figure 2. The values shown are for a single dilution (1:5) of the unknown sample. Based on the analysis of the various cytokines produced, the unknown samples can be grouped into Th_1, Th_2 or mixed responses as indicated.

Figure 3



Examples of Antigen Standard Curves for Individual Cytokines from the Plate Imaged

Figure 4



Bar graphs are shown for unknown samples #2 and #6 that show a strong Th_1 and Th_2 response, respectively. By analyzing multiple cytokines simultaneously within the same sample, Th_1 and Th_2 cytokine profiles can be easily and reliably identified.

Continued from page 7

differentiate between Th_1 and Th_2 cell populations (and even these may vary between mouse and human cells). Chemokine receptors such as CXCR3 and CCR5 are preferentially expressed in human Th_1 cells while Th_2 cells preferentially express CCR4 and CCR3.[36, 37] In mouse T cells, differentiated Th_2 cells were reported to express significantly more ICOS than differentiated Th_1 cells.[38] In the case of both the chemokine receptors and ICOS, however, these differences were quantitative and the same markers were observed on naïve T cells (albeit at different levels). In mice, the four transmembrane domain protein, Chandra, has been reported to be differentially expressed in Th_1 cells although the ligand and function of this protein remain unexplored.[39] Likewise, the IL-1 receptor family member, T1/ST2, has been reported to be preferentially expressed on the surface of mouse and human Th_2 cells and to be selectively secreted by activated Th_2 cells.[40, 41]

More recently, the T cell Ig domain, mucin domain 3 protein, Tim-3, has been reported to be selectively expressed on the surface of T-bet$^+$, Th_1 cells.[42, 43] Polarized Th_2 cells, in contrast, express higher levels of Tim-1[44] and Tim-2,[45] which has been proposed to regulate Th_2 responses.[45] Finally, mouse CD226 has been recently reported to be a specific marker expressed on the surface of Th_1 cells shown to regulate expansion and effector function in this cell population.[46]

Concluding Remarks

Taken together, existing studies suggest that expression of Notch ligands, as well as the cytokine milieu, can influence Th_1/Th_2 differentiation. Polarized Th_1 and Th_2 cells can differentially express transcription factors, cytokines, and cell surface markers that can be used to identify these divergent effector populations. BioLegend offers a wide array of antibodies for intracellular cytokine staining, cell surface receptor identification, Western blotting, single and multiplexed ELISAs for the investigation of Th_1 and Th_2 biology. Please see our website (www. biolegend. com Support section tab, Technical Protocols) for a detailed intracellular cytokine staining protocol and guide to cellular activation and protein transport inhibitors. These protocols are designed to optimize the detection of intracellular cytokines and gain information about the cellular phenotypes of the responding population. In addition, our website contains a general ELISA protocol and troubleshooting guide for the sensitive and accurate measurement of secreted cytokines as well as protocols for antibody cell surface staining and a comprehensive fluorochrome chart. In cases where the investigator has limited sample size, the Q-plex™ multiplexed Th_1/Th_2 ELISA offers an ideal format for the simultaneous analysis of nine different cytokines with a sample volume as little as 3–5 µl per well. An example of the plate image, standard curves, and data obtained with samples showing a clear Th_1 and Th_2 bias as well as those showing a more mixed response is shown beginning on page 8.

BioLegend Th_1- and Th_2-related Products

Specificity	Species Reactivity	Clone	Purified	LEAF™	Biotin	FITC	PE	PE/Cy5	PE/Cy5.5	PE/Cy7	APC	APC/Cy5.5	APC/Cy7	Alexa Fluor® 488	Alexa Fluor® 647	Alexa Fluor® 700	Pacific Blue™
Mouse CDs and Related Molecules																	
CD3	Mouse	17A2	X	X		X	X							X	X	X	X
CD3ε	Mouse	145-2C11	X	X	X	X	X	X	X	X	X	X		X	X		
CD4	Mouse	GK1.5	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CD4	Mouse	RM4-5	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CD4	Mouse	RM4-4	X			X	X										
CD8a	Mouse	53-6.7	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CD8a	Mouse	5H10-1	X			X											
CD8b	Mouse	CD8b	X		X	X	X										
CD25 (IL-2Rα)	Mouse	3C7	X	X			X										
CD25 (IL-2Rα)	Mouse	PC61	X	X	X	X	X	X		X	X			X	X	X	X
CD28	Mouse	37.51	X	X	X		X	X			X						
CD30	Mouse	mCD30.1	X	X	X		X										
CD44	Mouse	IM7	X	X	X	X	X	X			X			X	X	X	X
CD62L (L-selectin)	Mouse	MEL-14	X	X	X	X	X	X		X	X			X	X	X	X
CD80 (B7-1)	Mouse	16-10A1	X	X	X	X	X	X			X			X	X		
CD81 (TAPA-1)	Mouse	Eat-2	X	X	X		X										
CD86 (B7-2)	Mouse	GL-1	X	X	X	X	X	X		X	X			X	X	X	X
CD86 (B7-2)	Mouse	PO3	X	X	X	X	X	X		X	X			X	X	X	
IFN-γR β chain	Mouse	MOB-47	X				X										
CDw119 (IFN-γRα)	Mouse	2E2	X		X												
CD122 (IL-2Rβ)	Mouse	5H4	X	X	X		X										
CD134 (OX-40)	Mouse	OX-86	X		X												
CDw137 (4-1BB)	Mouse	17B5	X	X	X		X										
CDw137L (4-1BB Ligand)	Mouse	TKS-1	X	X	X		X										
CD150 (SLAM)	Mouse	TC15-12F12.2	X	X	X		X	X		X	X			X	X		
CD152 (CTLA-4)	Mouse	9H10	X	X													
CD152 (CTLA-4)	Mouse	UC10-4B9	X	X	X		X										
CD154 (CD40 Ligand)	Mouse	MR1	X	X	X		X										
CD195 (CCR5)	Mouse	HM-CCR5	X		X		X							X			
CD197 (CCR7)	Mouse	4B12	X		X		X	X			X			X	X		
CD210 (IL-10R)	Mouse	1B1.3a	X	X	X		X										
CD252 (OX40 Ligand)	Mouse	RM134L	X	X	X		X										
CD275 (ICOSL)	Mouse	HK5.3	X	X	X		X										
CD278 (ICOS)	Mouse	15F9	X		X		X	X									

Specificity	Species Reactivity	Clone	Purified	LEAF™	Biotin	FITC	PE	PE/Cy5	PE/Cy5.5	PE/Cy7	APC	APC/Cy5.5	APC/Cy7	Alexa Fluor® 488	Alexa Fluor® 647	Alexa Fluor® 700	Pacific Blue™
CD278 (ICOS)	Mouse	C398.4A	X	X	X	X	X				X			X	X		
CD279 (PD-1)	Mouse	RMP1-14	X	X													
CD279 (PD-1)	Mouse	RMP1-30	X		X		X										
CD284 (TLR4/MD2 Complex)	Mouse	MTS510	X	X	X		X										
GITR	Mouse	YGITR 765	X	X	X	X	X	X		X	X			X	X	X	
GITRL	Mouse	YGL 386	X		X		X										
IgE	Mouse	RME-1	X		X	X	X										
NF-κB p50	Mouse	Poly6197	X														
NF-κB p65	Mouse	Poly6226	X														
NOTCH1	Mouse	mN1A	X		X		X										
SOCS3	Mouse	SOC-25	X														
STAT 6	Mouse	Poly6252	X														
T-bet	Mouse	Poly6235	X														
Tim-1	Mouse	RMT1-4	X		X		X										
Tim-2	Mouse	RMT2-1	X		X												
Tim-3	Mouse	RMT3-23	X				X										
Mouse MHC Antigens																	
I-A/I-E	Mouse	M5/114.15.2	X	X	X	X	X	X			X			X	X	X	X
I-A^b	Mouse	AF6-120.1	X		X	X	X							X	X		
I-A^b	Mouse	KH74	X		X	X								X	X		
I-A^b ($A\beta^b$)	Mouse	25-9-17	X		X	X								X	X		
I-A^d	Mouse	39-10-8	X		X	X								X	X		
I-A^k ($A\alpha^k$)	Mouse	11-5.2	X		X	X	X							X	X		
I-A^k ($A\beta^k$)	Mouse	10-3.6	X		X	X	X							X	X		
I-A^q	Mouse	KH116	X		X									X	X		
I-E^k	Mouse	14-4-4S	X		X	X	X							X	X		
Mouse T Cell Receptors (TCRs)																	
β T Cell Receptor	Mouse	H57-597	X	X	X	X	X	X			X			X	X		
Mouse Cytokines/Chemokines																	
IL-2	Mouse	JES6-1A12	X	X												X	
IL-2	Mouse	JES6-5H4	X	X	X	X	X				X			X	X	X	
IL-3	Mouse	MP2-8F8	X	X			X										
IL-3	Mouse	MP2-43D11			X												
IL-4	Mouse	11B11	X	X			X				X			X	X		
IL-4	Mouse	BVD6-24G2			X												
IL-5	Mouse	TRFK5	X	X			X				X						
IL-5	Mouse	TRFK4			X												
IL-6	Mouse	MP5-20F3	X	X			X										
IL-6	Mouse	MP5-32C11			X												
IL-10	Mouse	JES5-2A5	X	X	X												

Specificity	Species Reactivity	Clone	Purified	LEAF™	Biotin	FITC	PE	PE/Cy5	PE/Cy5.5	PE/Cy7	APC	APC/Cy5.5	APC/Cy7	Alexa Fluor® 488	Alexa Fluor® 647	Alexa Fluor® 700	Pacific Blue™
Mouse Cytokines/Chemokines (continued)																	
IL-10	Mouse	JES5-16E3	X	X	X	X	X				X			X	X		
IL-12/IL-23 (p40)	Mouse	C15.6	X	X			X				X						
IL-12/IL-23 (p40)	Mouse	C17.8		X	X						X						
IL-17	Mouse	TC11-18H10.1	X				X										
IL-17	Mouse	TC11-8H4			X												
GM-CSF	Mouse	MP1-22E9	X	X		X	X										
GM-CSF	Mouse	MP1-31G6	X		X												
IFN-γ	Mouse	R4-6A2	X	X	X												
IFN-γ	Mouse, Rat	DB-1	X	X		X	X										
IFN-γ	Mouse	XMG1.2	X	X	X	X	X				X			X	X	X	
TNF-α	Mouse	MP6-XT22	X	X	X	X	X				X			X	X	X	
TNF-α	Mouse	TN3-19.12	X	X			X										
TNF-α	Mouse	Poly5062			X												
TNF-α	Mouse	6B8	X	X													
Rat CDs and Related Molecules																	
CD3	Rat	1F4				X											
CD4	Rat	W3/25	X		X	X	X				X						
CD8a	Rat	OX-8	X			X	X										
CD8a	Rat	G28	X	X	X	X	X				X						
CD8b	Rat	341	X	X	X	X											
CD25 (IL-2Rα)	Rat	OX-39	X			X	X										
CD28	Rat	JJ319	X	X		X	X				X						
CD62L (L-Selectin)	Rat	OX-85	X		X	X								X	X		
CD80 (B7-1)	Rat	3H5	X	X	X		X										
CD81 (TAPA-1)	Rat	Eat-2	X	X	X		X										
CD86 (B7-2)	Rat	24F	X	X	X	X	X										
CD152 (CTLA-4)	Rat	WKH203	X		X	X								X	X		
CD252 (OX-40 Ligand)	Rat	ATM-2	X	X	X		X										
CD278 (ICOS)	Rat	C398.4A	X	X	X	X	X				X			X	X		
NF-κB p50	Rat	Poly6197	X														
NF-κB p65	Rat	Poly6226	X														
RT1D (MHC class II)	Rat	14-4-4S	X		X	X	X							X	X		
STAT 6	Rat	Poly6252	X														
Rat T Cell Receptors (TCRs)																	
α/β T Cell Receptor	Rat	R73	X	X	X	X	X				X						
Rat Cytokines/Chemokines																	
IL-2	Rat	BL-7015	X														
IL-2	Rat	BL-7030			X												
IL-4	Rat	BL-7045	X														

Specificity	Species Reactivity	Clone	Purified	LEAF™	Biotin	FITC	PE	PE/Cy5	PE/Cy5.5	PE/Cy7	APC	APC/Cy5.5	APC/Cy7	Alexa Fluor® 488	Alexa Fluor® 647	Alexa Fluor® 700	Pacific Blue™
IL-4	Rat	BL-7060			X												
IFN-γ	Rat	DB-1	X	X		X	X										
IFN-γ	Rat	Poly5109			X												
TNF-α	Rat	TN3-19.12	X	X			X										
TNF-α	Rat	Poly5062			X												
Human CDs and Related Molecules																	
CD3 (T3)	Human	HIT3a	X	X	X	X	X	X		X	X		X	X	X	X	
CD3 (T3)	Human	UCHT1	X	X	X	X	X	X		X	X			X	X	X	X
CD4 (T4)	Human	RPA-T4	X	X	X	X	X	X		X	X		X	X	X	X	X
CD8a (T8)	Human	HIT8a	X		X	X	X	X		X	X			X	X	X	
CD8a (T8)	Human	RPA-T8	X	X	X	X	X	X	X	X	X		X	X	X	X	X
CD25 (IL-2Rα)	Human	BC96	X			X	X	X		X	X		X	X	X	X	X
CD26	Human	BA5b	X			X	X	X									
CD27	Human	O323	X		X	X	X				X				X	X	
CD28 (T44, Tp44)	Human	CD28.2	X	X	X	X	X	X			X			X	X	X	
CD44 (Hermes, Pgp-1)	Human	IM7	X	X	X	X	X	X			X			X	X	X	X
CD62L (L-Selectin)	Human	DREG-56	X	X		X	X	X			X		X	X	X	X	
CD80 (B7-1)	Human	2D10	X	X	X	X	X	X						X	X		
CD86 (B7-2)	Human	IT2.2	X	X	X		X	X			X			X	X		X
CDw119 (IFN-γRα)	Human	GIR-208	X	X			X										
CDw119 (IFN-γRα)	Human	GIR-94	X		X		X										
IFN-γ R β chain	Human	2HUB-159	X	X			X										
CDw137 (4-1BB)	Human	4B4-1	X				X										
CDw137L (4-1BBL)	Human	5F4	X				X										
CD150 (SLAM)	Human	A12 (7D4)	X	X	X	X	X							X	X		
CD154 (CD40 Ligand)	Human	24-31	X	X	X	X	X	X			X			X	X	X	X
CD184 (CXCR4, Fusin)	Human	12G5	X	X	X		X	X			X						
CD193 (CCR3)	Human	5E8	X				X										
CD195 (CCR5)	Human	HEK/1/85a	X		X	X	X							X	X	X	
CD195 (CCR5)	Human	T21/8	X	X	X		X										
CD195 (CCR5) Non-Phosphorylated (Ser337)	Human	RC-10	X		X												
CD195 (CCR5) Phosphorylated (Ser337)	Human	V14/2	X		X												
CD195 (CCR5) Phosphorylated (Ser349)	Human	E11/19	X		X												
CD210 (IL-10R)	Human	3F9	X	X			X										
CDw218a (IL-18Rα)	Human	H44	X	X	X		X										
CD226 (DNAM-1)	Human	DX11	X			X								X	X		
CD275 (ICOSL)	Human	2D3	X		X		X										
CD278 (ICOS)	Human	C398.4A	X	X	X	X	X				X			X	X		
CD282 (TLR2)	Human	TL2.1	X	X	X	X	X							X	X		
CD284 (TLR4)	Human	HTA125	X	X	X		X										

Specificity	Species Reactivity	Clone	Purified	LEAF™	Biotin	FITC	PE	PE/Cy5	PE/Cy5.5	PE/Cy7	APC	APC/Cy5.5	APC/Cy7	Alexa Fluor® 488	Alexa Fluor® 647	Alexa Fluor® 700	Pacific Blue™
Human CDs and Related Molecules (continued)																	
GATA 3	Human	Poly6071	X											X			
GITR (AITR)	Human	621	X	X			X										
GITR Ligand (AITRL)	Human	EB11-2	X	X			X										
NF-κB p50	Human	4D1	X											X			
NF-κB p50	Human	Poly6197	X														
NF-κB p65	Human	Poly6226	X														
NOTCH1	Human	mN1A	X		X		X										
SOCS3	Human	SOC-25	X														
STAT 4	Human	Poly6100	X														
STAT 6	Human	Poly6252	X														
T-bet	Human	Poly6235	X														
Human MHC Antigens																	
HLA-A,B,C (MHC Class I)	Human	W6/32	X	X		X	X	X			X			X	X		X
HLA-DR (MHC Class II)	Human	L243	X	X	X	X	X	X		X	X		X	X	X	X	X
Human T Cell Receptors (TCRs)																	
α/β T Cell Receptor (α/β TCR)	Human	IP26	X		X	X	X	X						X	X		
α/β T Cell Receptor (α/β TCR)	Human	T10B9	X			X											
Multicolor Cocktail Reagents																	
CD3 FITC/CD4 PE Cocktail	Human	UCHT1/RPA-T4				X	X										
CD3 FITC/CD8 PE Cocktail	Human	UCHT1/RPA-T8				X	X										
CD3 PE-Cy5/CD4 PE/CD8 FITC Cocktail	Human	UCHT1/RPA-T4/RPA-T8				X	X	X									
CD4 PE-Cy5/CD25 PE Cocktail	Human	RPA-T4/BC96					X	X									
Human Cytokines/Chemokines																	
IL-2	Human	MQ1-17H12	X	X		X	X				X			X	X	X	
IL-2	Human	Poly5111			X												
IL-3	Human	BVD3-1F9	X		X		X										
IL-3	Human	BVD8-3G11	X	X													
IL-4	Human	8D4-8	X	X			X										
IL-4	Human	MP4-25D2	X	X	X	X	X				X			X	X		
IL-5	Human	JES1-39D10	X	X			X										
IL-5	Human	JES1-5A10	X	X	X												
IL-5	Human	TRFK5	X	X			X				X						
IL-6	Human	MQ2-13A5	X	X		X	X				X						
IL-6	Human	MQ2-39C3	X		X												
IL-10	Human	JES3-12G8	X		X												
IL-10	Human	JES3-19F1	X	X			X				X						
IL-10	Human	JES3-9D7	X	X			X				X			X	X		
IL-12/IL-23 (p40)	Human	C8.3	X	X													

Specificity	Species Reactivity	Clone	Purified	LEAF™	Biotin	FITC	PE	PE/Cy5	PE/Cy5.5	PE/Cy7	APC	APC/Cy5.5	APC/Cy7	Alexa Fluor® 488	Alexa Fluor® 647	Alexa Fluor® 700	Pacific Blue™
IL-12/IL-23 (p40)	Human	C8.6		X	X												
IL-12/IL-23 (p40)	Human	C11.5	X	X		X	X				X						
IL-12 (p70)	Human	7B12	X	X													
IL-13	Human	JES10-5A2	X	X			X				X						
IL-13	Human	Poly5020			X												
IL-23 (p19)	Human	HLT2736	X		X												
GM-CSF	Human	BVD2-23B6	X	X													
GM-CSF	Human	BVD2-21C11	X		X		X										
IFN-γ	Human	NIB42	X	X													
IFN-γ	Human	4S.B3	X		X	X	X				X			X	X	X	
IFN-γ	Human	MD-1	X	X													
IFN-γ	Human	B27	X	X		X	X				X					X	
TNF-α	Human	MAb1	X	X													
TNF-α	Human	MAb11	X		X	X	X				X			X	X		
TNF-β	Human	359-238-8	X	X													
TNF-β	Human	359-81-11	X	X	X		X										
Related Products																	
Cytokine ELISA MAX™ Sets																	
Q-Plex™ Cytokine Array Kits																	

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