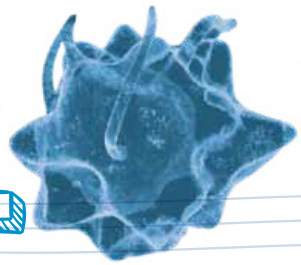
BD

I attack invaders **outside** the cells.

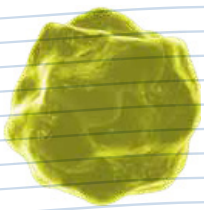
T CELL



B&T 細胞攻略

流式細胞儀應用

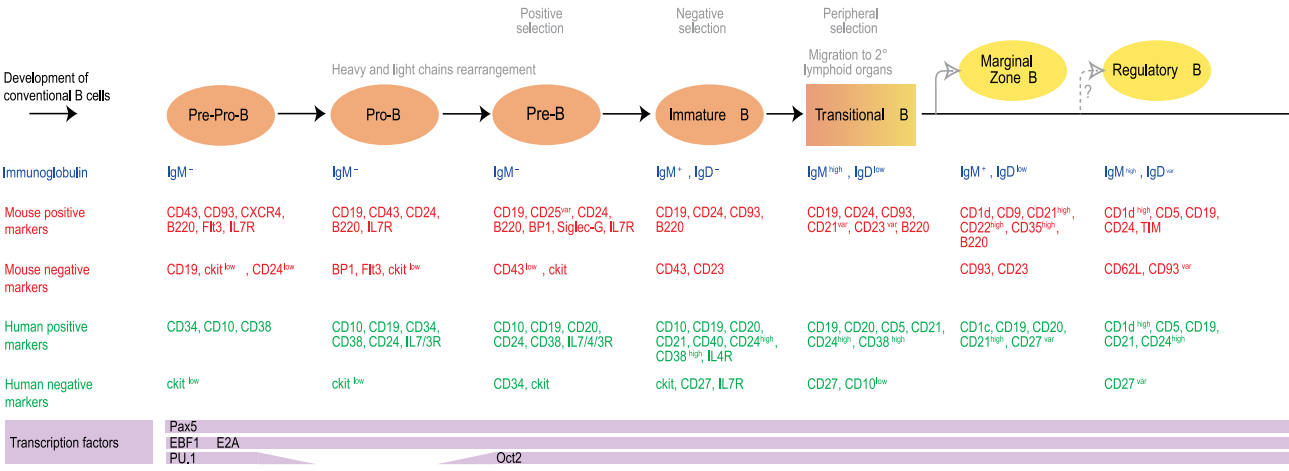
I attack invaders **inside** the cells.



B CELL

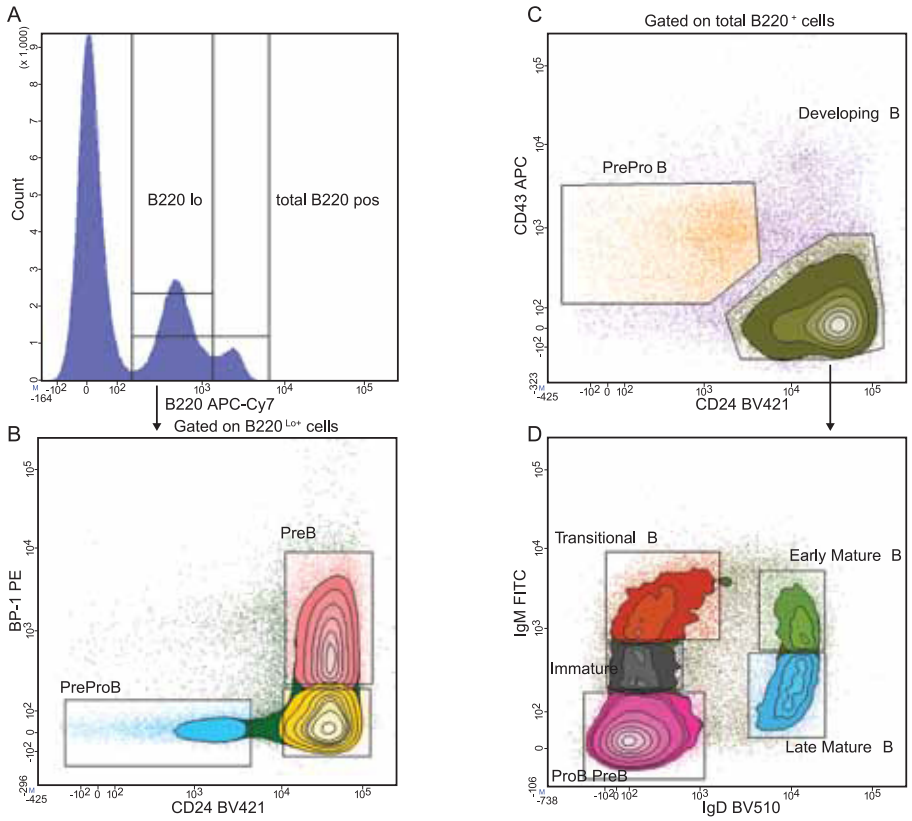
B CELL

Summary of the key developmental stages and markers of B cells

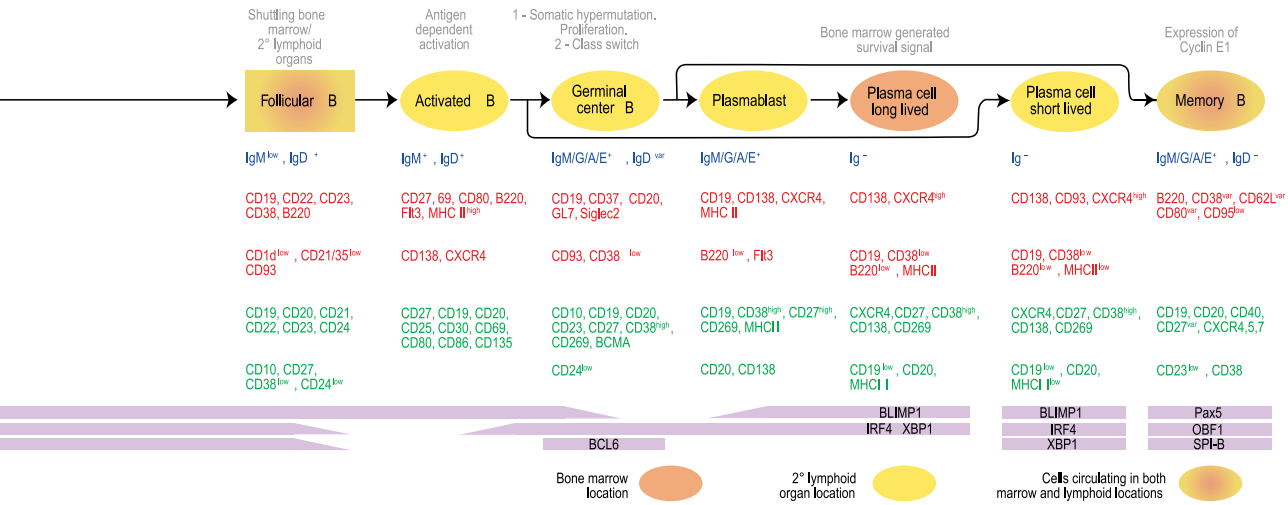


Immunoglobulin	IgM⁻	IgM⁻	IgM⁻	IgM⁺, IgD⁻	IgM^{high}, IgD^{low}	IgM⁺, IgD^{low}	IgM^{high}, IgD^{var}
Mouse positive markers	CD43, CD93, CXCR4, B220, Flt3, IL7R	CD19, CD43, CD24, B220, IL7R	CD19, CD25^{var}, CD24, B220, BP1, Siglec-G, IL7R	CD19, CD24, CD93, B220	CD19, CD24, CD93, CD21^{var}, CD23^{var}, B220	CD1d, CD9, CD21^{high}, CD22^{high}, CD35^{high}, B220	CD1d^{high}, CD5, CD19, CD24, TIM
Mouse negative markers	CD19, ckit^{low}, CD24^{low}	BP1, Flt3, ckit^{low}	CD43^{low}, ckit	CD43, CD23		CD93, CD23	CD62L, CD93^{var}
Human positive markers	CD34, CD10, CD38	CD10, CD19, CD34, CD38, CD24, IL7/3R	CD10, CD19, CD20, CD24, CD38, IL7/4/3R	CD10, CD19, CD20, CD21, CD40, CD24^{high}, CD38^{high}, IL4R	CD19, CD20, CD5, CD21, CD24^{high}, CD38^{high}	CD1c, CD19, CD20, CD21^{high}, CD27^{var}	CD1d^{high}, CD5, CD19, CD21, CD24^{high}
Human negative markers	ckit^{low}	ckit^{low}	CD34, ckit	ckit, CD27, IL7R	CD27, CD10^{low}		CD27^{var}
Transcription factors	Pax5						
	EBF1 E2A						
	PU.1		Oct2				

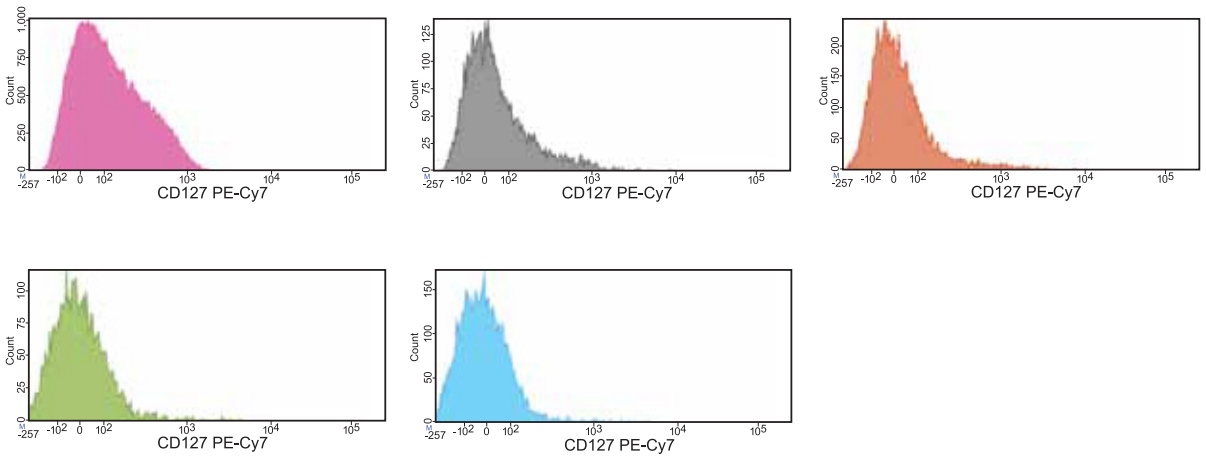
實驗範例



B 細胞是免疫系統的重要角色，近來發現 **B-cell** 發育或功能與罕見疾病的成因相關，例如：多發性硬化症 **(multiple sclerosis)**、紅斑性狼瘡 **(systemic lupus erythematosus)**、第一型糖尿病 **(Type I Diabetes)**、類風濕性關節炎 **(rheumatoid arthritis)**、**B cell** 淋巴癌等，**B** 細胞目前為新興疫苗及細胞療法研究方向。在與抗原接觸的前後，**B** 細胞有一系列的發育時期，各期的細胞都有其特殊功能、細胞標誌、轉錄因子等



E CD127 expression on Pro-B/Pre-B, Immature Transitional, Early Mature, and Late Mature B cells



Analysis of B-cell developmental stages in mouse bone marrow

C57BL/6 mouse bone marrow cells were stained with the following fluorescent antibodies: IgM FITC, CD43 APC, BP-1 PE, CD127 PE-Cy™7, CD45R/B220 APC-Cy7, CD24 BD Horizon™ Brilliant Violet™ 421 (BV421), and IgD BD Horizon™ Brilliant Violet™ 510 (BV510), and analyzed using a BD FACSCanto™ II flow cytometer.

Immunophenotyping

B 細胞表型可用多色染色技術來偵測，以下是 BD Biosciences 建議的實驗設計：

Backbone Markers

基本會表現在大部分 B 細胞的 Key Marker 如右表

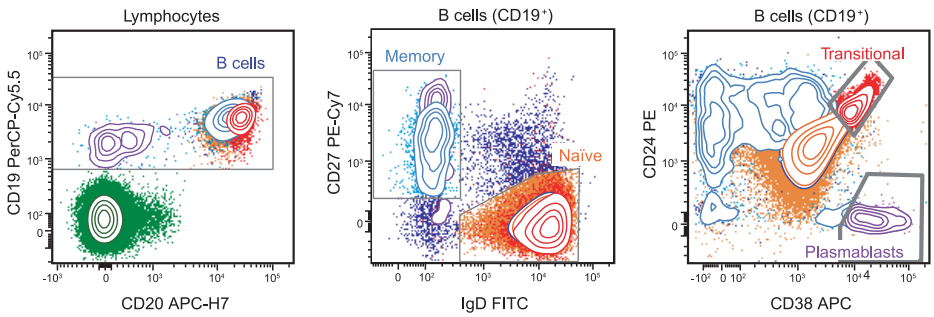
Human	Mouse
CD19	CD45R/B220

Basic Panel

利用 CD19, CD20, IgD, CD27, CD38 和 CD24 這 6 個細胞標誌來辨識不同的 B 細胞族群。

Human B-Cell Subset	Marker
Transitional B cells	$CD24^{high}$, $CD38^{high}$
Naive B cells	$CD27^{-}$, IgD^{+}
Memory B cells	$CD27^{+}$, IgD^{-}
Plasmablasts	$CD38^{+}$

實驗範例



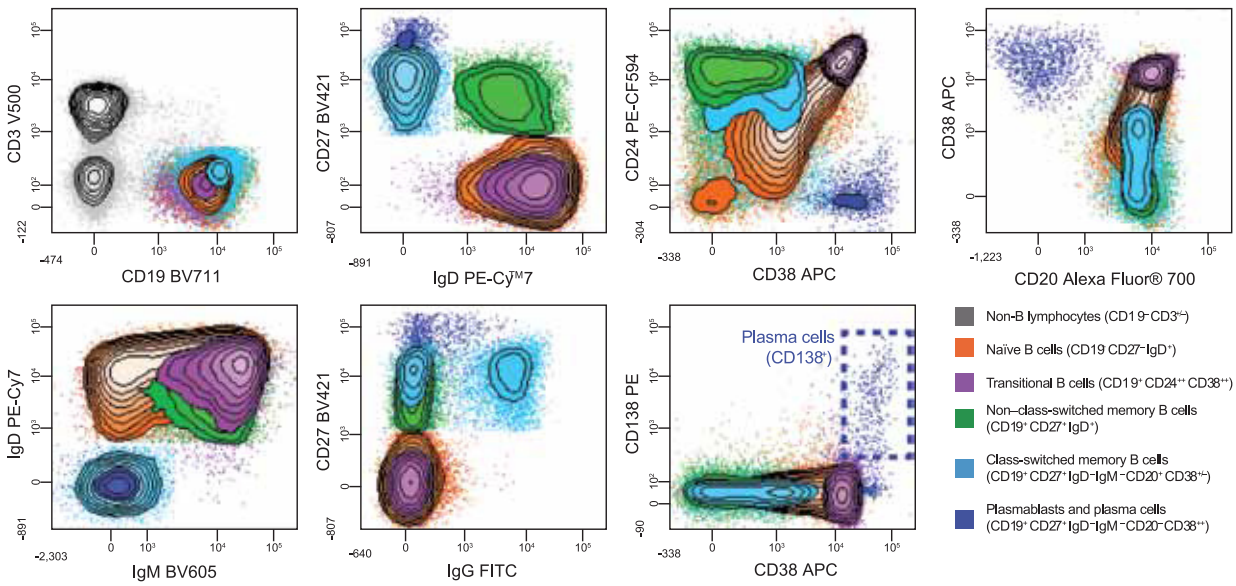
Six-color analysis of human peripheral blood cells

Human peripheral blood mononuclear cells (PBMCs) were stained with the following fluorescent antibodies : CD19 PerCP-Cy™5.5, CD20 APC-H7, IgD FITC, CD27 PE-Cy™7, CD38 APC, and CD24 PE, and analyzed using a BD FACSVerse™ flow cytometer.

Deeper Phenotyping

另外可加入 Chemokine receptor, CD62L 及 Activation Marker CD69, CD25, CD80, CD86 等細胞標誌來探討特異的免疫反應。

實驗範例



Ten-color analysis of human peripheral blood cells

Human PBMCs were stained with the following fluorescent antibodies: CD19 BD Horizon™ Brilliant Violet™ 711, CD20 Alexa Fluor® 700, IgD PE-Cy7, CD27 BV421, CD38 APC, CD24 BD Horizon™ PE-CF594, CD3 BD Horizon™ V500, CD138 PE, IgM BD Horizon™ Brilliant Violet™ 605 (BV605), and IgG FITC, and analyzed using a BD LSRFortessa™ flow cytometer.

Intracellular Cytokine

偵測 B 細胞所分泌的免疫球蛋白 (Immunoglobulin) 和細胞激素 (Cytokine) 種類、濃度，以了解他們在疾病病程中的所產生的免疫反應變化。

Cytokine-producing B cells

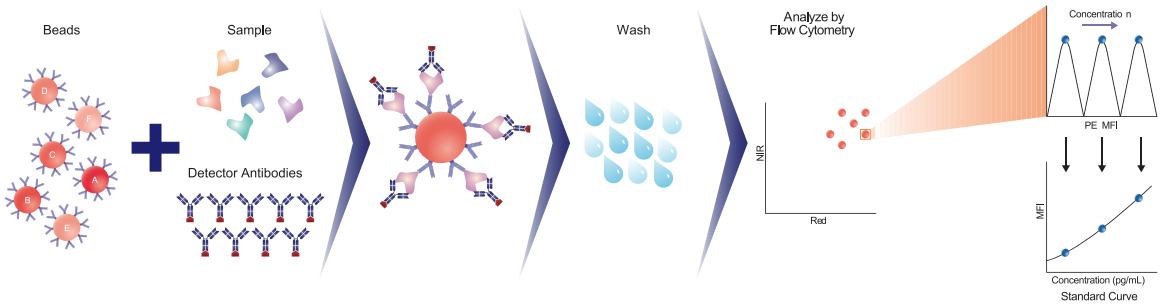
Cell type	Cytokines Secreted
B effector 1 (Be-1)	IFN-γ, IL-12, TNF
B effector 2 (Be-2)	IL-2, IL-4, IL-6, TNF
Regulatory B	IL-10, TGF-β1

強力推薦

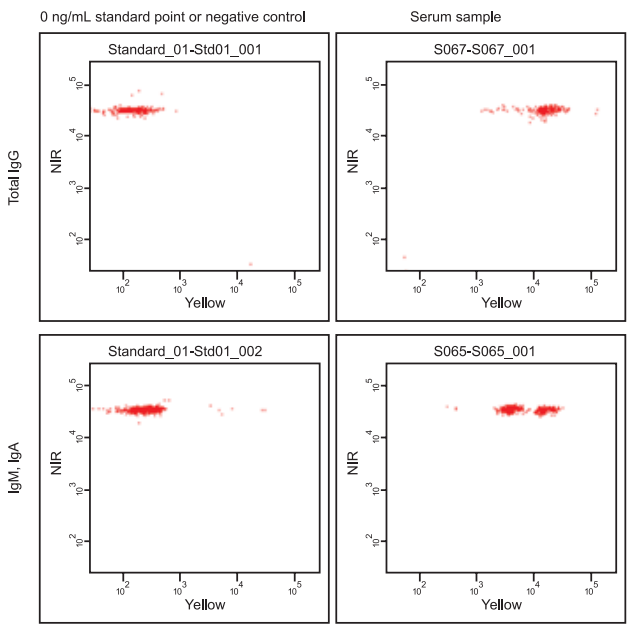
BD™ Cytometric Bead Array (CBA)

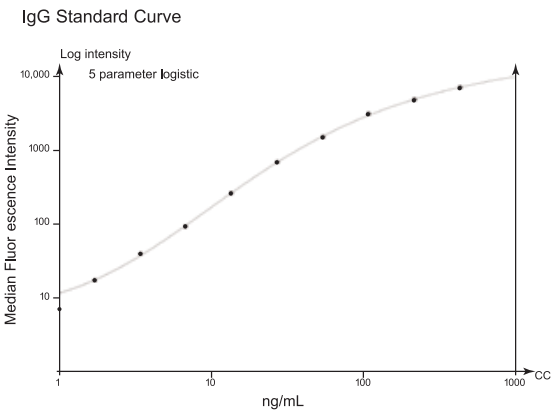
可快速大量定性或定量免疫球蛋白 (Immunoglobulin) 和細胞激素 (Cytokine) 的流式細胞儀技術

BD CBA 是種結合流式細胞儀螢光偵測和微球免疫分析的應用技術，可以輕鬆地偵測多種蛋白濃度。其作用原理為跟 ELISA 類似，為 Sandwich 法。就是利用大小相同、螢光強度不同的微球，上面帶有可以抓附特定蛋白的抗體 (capture antibody)，與樣本 (例如血清、血漿、培養上清液、細胞萃取液等) 及 PE 偵測抗體 (Detection Antibody) 作用後，以流式細胞儀進行分析。藉由 PE 平均螢光強度 (MFI) 的不同，可以計算出樣本內特定蛋白的含量。



實驗範例





	Total IgG	IgM	IgA
Donor 1	16.9	1.3	1.6
Donor 2	10.9	2.4	0.9
Donor 3	8.9	0.7	1.5
Donor 4	14.6	2.8	1.5
Donor 5	11.6	0.3	1.1
Expected Range	7–14	0.5–3.3	0.8–3.9

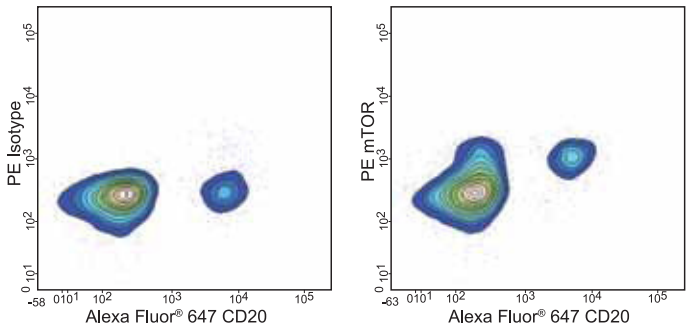
Quantitation of immunoglobulins in human serum samples

Serum from five donors was tested using BD™ CBA Human Immunoglobulin Flex Sets to analyze total IgG, IgM, and IgA. Data was acquired on a BD FACSArray™ flow cytometer and analyzed using FCAP Array software. Dot plots for a negative control (detector alone) and one of the samples are shown. The results calculated (in g/L) on the basis of standard curves are shown, as well as the expected range.

Intracellular Signaling

B 細胞發育、活化與分化時期，細胞內的轉錄因子 (Transcription factor)、磷酸化蛋白 (phosphosignaling proteins)、細胞激素 (cytokines) 等蛋白會隨之變化。

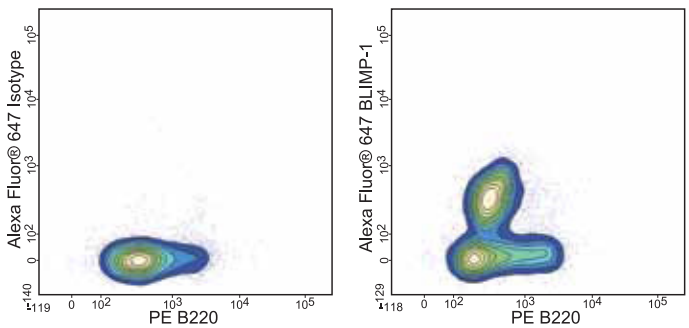
Detection of Transcription Factors



Phospho-mTOR staining of human cells

Human peripheral B lymphocytes were stimulated with CpG ODN2395, then fixed with BD Cytofix™ fixation butter, permeabilized using BD Phosflow™ perm buffer III, and stained with anti-mTOR (pS2448) PE or a matching isotype control, and anti-CD20 Alexa Fluor® 647. Cells were analyzed using a BD FACSCanto™ II system.

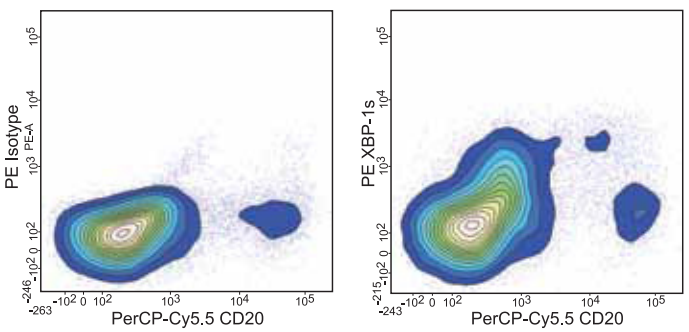
Detection of Phosphoproteins



Blimp-1 staining in activated mouse splenocytes

B6 mouse splenocytes activated with LPS for 3 days were analyzed using the BD Pharmingen transcription factor buffer set, anti-CD45R/B220 PE, and either anti-Blimp-1 Alexa Fluor® 647 or a matching isotype control. Flow cytometry was performed using a BD FACSCanto II system.

Detection of Cytokines



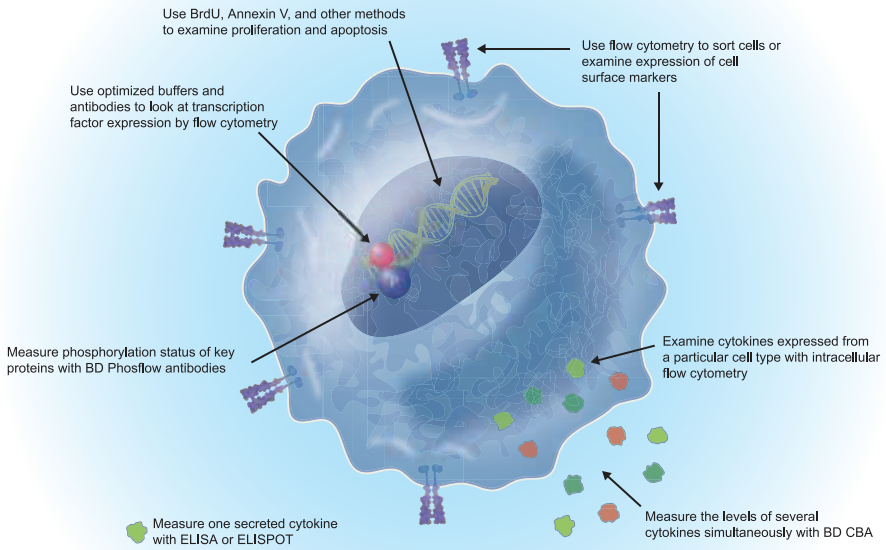
XBP-1s expression in CpG-stimulated human PBMCs

CpG-stimulated human PBMCs were incubated with BD Horizon™ fixable viability stain 450, fixed and permeabilized using the BD Pharmingen transcription factor buffer set, and stained with anti-CD20 PerCP-Cy™5.5, and either anti-XBP-1s PE or a matching isotype control. Flow cytometry was performed using a BD FACSCanto II system.

T CELL

T 細胞是淋巴細胞之一，在胸腺內分化成熟後移至周圍淋巴組織中，主要在免疫反應中扮演著重要的角色。依據 **T** 細胞表面標誌、功能的差異可以分成不同亞群的 **T** 細胞，可用流式細胞儀等研究技術鑑定 **T** 細胞分型和分泌之細胞激素，此為主要的免疫研究趨勢。

目前可用於 T 細胞研究的免疫實驗技術整理及比較



Comparison of technologies for T cell research

Tool/Technology	Flow Cytometry/Surface	Flow Cytometry/Intracellular	BD Cytometric Bead Array (CBA)	ELISPOT	ELISA	In Vivo Capture Assay
Molecules detected	Surface	Intracellular and surface	Secreted or intracellular	Secreted (in situ)	Secreted	Secreted (in vivo)
Multiparameter	Yes	Yes	Yes	No	No	No
Single cell/cell subset information	Yes	Yes	No	Frequencies, no subset information	No	No
Antigen specifi c	Yes	Yes	Yes	Yes	Yes	Yes
Post-assay viability	Yes	No	Yes, for secreted molecules	No	Yes	Yes
Quantitation of protein	Possible*	Possible*	Yes	No	Yes	Yes
Instrumentation	Flow cytometer	Flow cytometer	Flow cytometer	ELISPOT reader	Spectrophotometer	Spectrophotometer

*With a standard such as BD Quantibrite™ beads

Immunophenotyping

T 細胞根據其功能可分成三大類：

毒殺性 T 細胞 (Cytotoxic T cell / Tc)

Tc 細胞可以透過分泌穿孔素 (perforin)、顆粒脢 (granzymes)、顆粒溶解素 (granulysin) 等來殺死感染目標細胞。

輔助型 T 細胞 (Helper T cell / Th)

Th 細胞可經由些微細胞毒性及細胞激素影響其他細胞，例如：B 細胞，巨噬細胞 (macrophage)、嗜酸性球 (eosinophil)、嗜中性球 (neutrophil) 等來清理病原體。

調節性 T 細胞 (Regulatory T cell / Treg)

Treg 細胞可藉由調節作用 T 細胞 (effector T cell) 分泌細胞激素等機制來抑制其他 T 細胞的功能。因此，Treg 細胞在維持免疫恆定中扮演十分重要的角色。$CD4^+$ Treg 分成兩種，一為 Natural Treg ，簡稱 nTreg，持續表現 CD25 及 FoxP3；另一種為 Induce Treg，簡稱 iTreg，CD25 及 FoxP3 是在活化後才表現。

Major Known T-Cell Markers

Type of Cell	Cytotoxic	Th1	Th2	Th9[5]	Th17	Tfh[6]	Treg
Main Function	Kill virus-infected cells	Activate microbicidal function of infected macrophages, and help B cells to produce antibody	Help B cells and switch antibody isotype production	T cell proliferation and enhanced IgG and IgE production by B cells	Enhance neutrophil response	Regulate development of antigen specific B cell development and antibody production	Immune regulation
Pathogens Targeted	Viruses and some intracellular bacteria	Intracellular pathogens	Parasites	Parasites	Fungi and extracellular bacteria		
Harmful Function	Transplant rejection	Autoimmune disease	Allergy, asthma	Allergy	Organ-specific autoimmune disease	Autoimmune disease	Autoimmune disease, cancer
Extracellular Markers	CD8	CD4 CXCR3	CD4 CCR4, Crth2 (human)	CD4	CD4, CCR6	CD4, CXCR5	CD4, CD25
Differentiation Cytokines		IFN-γ, IL-2, IL-12, IL-18, **IL-27**	IL-4, IL-2, **IL-33**	IL-4, **TGF-β**	**TGF-β**, IL-6, IL-1, IL-21, IL-23	IL-12, IL-6	**TGF-β**, IL-12
Effector Cytokines	IFN-γ, TNF, LT-α	IFN-γ, LT-α, TNF	IL-4, IL-5, IL-6, IL-13	IL-9, IL-10	IL-17A, IL-17F, IL-21, **IL-22**, IL-26, TNF, CCL20	IL-21	**TGF-β**, IL-10
Transcription Factors		T-bet, Stat1, Stat6	GATA3, Stat5, Stat6	GATA3, Smads, Stat6	RORγt, RORα, Stat3	Bcl-6, MAF	FoxP3, Smad3, Stat5

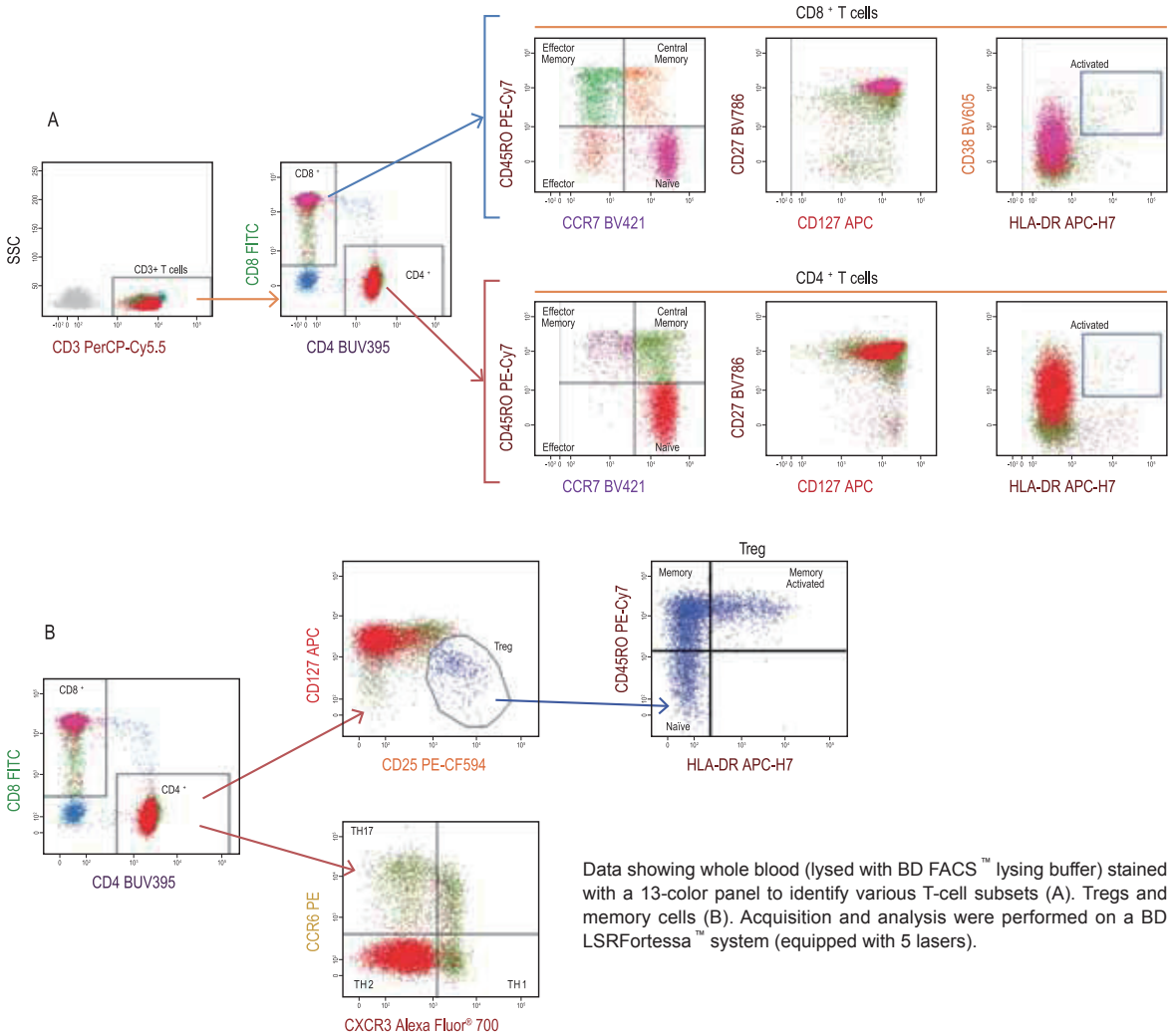
Markers can be altered as a result of cellular environment, differentiation state, and other factors. Key cytokines appear in bold. BD Biosciences offers reagents for molecules that are red.

實驗範例

Marker	Fluorochrome	Purpose
Viability dye	V500	Viability
CD3	PerCP-Cy5.5	T cell marker
CD4	BUV395	T cell subsetting
CD8	FITC	T cell subsetting
CD127	Alexa Fluor® 647	Regulatory T Cell Marker
HLA-DR	APC-H7	Activation
CD45RO	PE-Cy7	Memory
CD197 (CCR7)	BV421	Naïve/Memory
CD38	BV605	Activation
CD27	BV786	Memory
CD25	PE-CF594	Regulatory T Cell Marker/Activation
CD196 (CCR6)	PE	Th17 Cell Marker
CXCR3	Alexa Fluor® 700	Th1 Cell Marker

Phenotyping of Cells with Unique Surface Profiles

The 13-color panel below examines memory and activation status of multiple T-cell subsets



Data showing whole blood (lysed with BD FACS™ lysing buffer) stained with a 13-color panel to identify various T-cell subsets (A). Tregs and memory cells (B). Acquisition and analysis were performed on a BD LSRFortessa™ system (equipped with 5 lasers).

Intracellular Cytokine

常見的流式細胞儀實驗技術中，可用來偵測細胞激素的方式除了上述介紹的 BD CBA 外，另外還有 Intracellular Staining (ICS)。T 細胞的研究中，通常會結合這兩種方式進行不同 T 細胞亞型細胞激素的大量樣本定量偵測，以加速研究進行。

CBA 與 ICS 兩種技術的比較

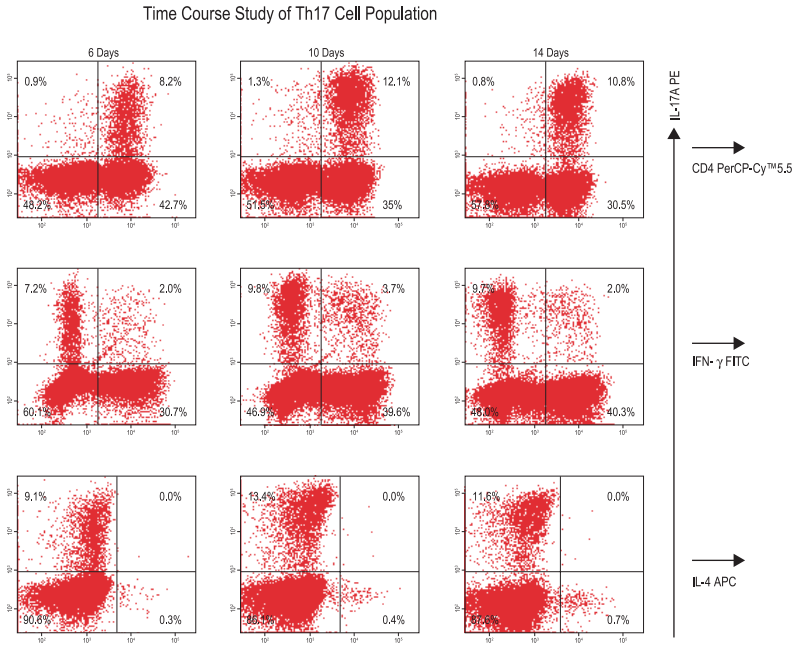
CAPABILITY	CBA	ICS
Allows detection of multiple cytokines in same experiment	√	√
Can obtain phenotype of specific cells expressing cytokine of interest		√
Can measure quantity of cytokine secreted	√	

實驗範例

Combining CBA and Intracellular Flow Cytometry to Examine Th17-cell Differentiation

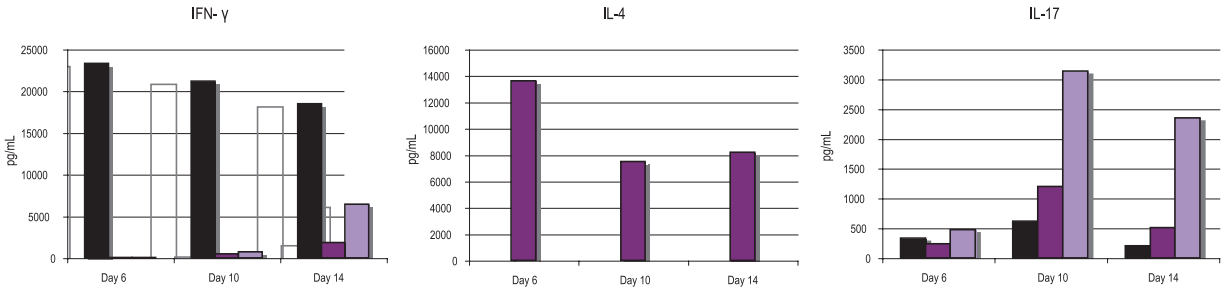
With both CBA and intracellular cytokine staining (ICS) available, scientists at BD performed an experiment to examine T-cell differentiation, which can be induced by activation and treatment with cytokines. To study Th1/ Th2/Th17 cell differentiation, CD4+-panned human T cells isolated from normal donors were co-stimulated with CD3/CD28 and:

- IL-2, IL-12, and a neutralizing mAb to IL-4 (Th1 polarization)
- IL-2, IL-4, and a neutralizing mAb to IFN-γ (Th2 polarization)
- IL-2, IL-6, IL-1β, TGF-β, IL-23, and a neutralizing mAb to IL-4 and IFN-γ (also tested with and without IL-2, IL-6, and TGF-β) (Th17 polarization)



Representative data from Th17 polarized cell ICS experiments comparing levels of IL-17A with CD4, IFN-γ, and IL-4.

Cells were treated under the Th17 polarizing conditions described for the indicated time points. They were treated with BD GolgiStop (monensin) inhibitor, fixed and permeabilized with BD Cytofix/Cytoperm buffer, and then stained with antibodies against the indicated cytokines. At 6 days there were signifi cant numbers of cells expressing IL-17A, with numbers of cells increasing at day 10 and then leveling off.

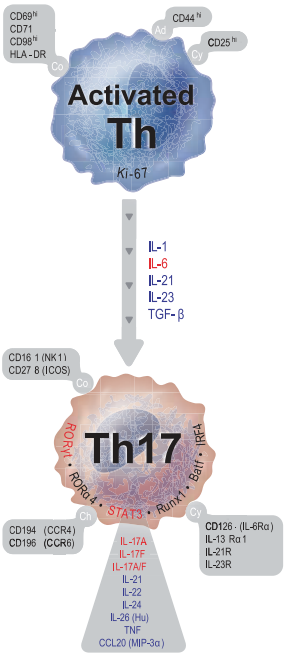


Data comparing cytokine levels as a result of different polarization conditions.

Supernatants from cells were polarized toward a Th1, Th2, or Th17 phenotype and cytokine levels were measured by CBA. As anticipated, each polarized condition resulted in the production of the signature cytokine associated with each Th cell type.

Intracellular Signaling

複雜的訊息傳遞分子包括了抗原受器、細胞激素及轉錄因子等，在免疫系統啟動時，會傳遞訊息到 Adaptive immune system 進而調控 T 細胞活化、分化，例如，Naïve CD4 T 細胞會分化成 Th1, Th2, Th9, Th17, Tfh 或 Tregs。



Signaling pathway for Th17 cell differentiation

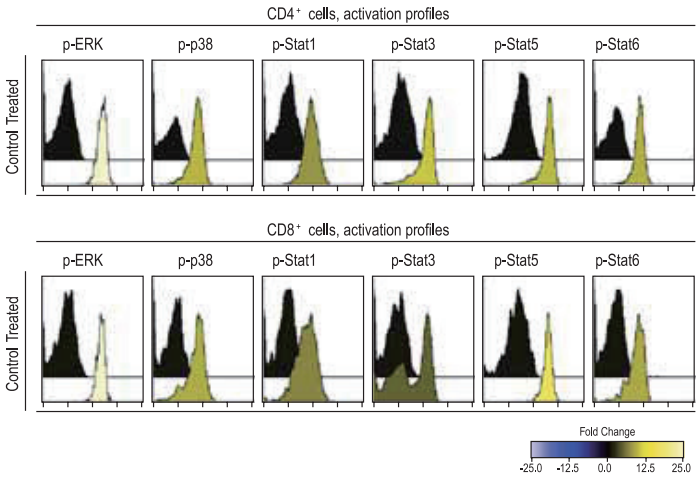
IL-6 stimulation leads to the phosphorylation at STAT3. STAT3 in concert with ROR γ T induced IL-17, the signature cytokine of Th17 cells.

強力推薦

BD™ Phosflow 有效偵測分析 T 細胞訊息傳遞路徑的流式細胞儀實驗技術

2002 年，美國史丹佛大學 Garry Nolan 博士研發出跨時代的研究方式：結合磷酸化專一抗體及流式細胞儀技來進行磷酸化蛋白的研究。流式細胞儀技術只需要少量的樣本，就可以進行單一細胞或是各分群細胞的定量多項分析。身為這項技術的世界領導品牌 "BD Biosciences" 和 Garry Nolan 博士共同發展了 "BD™ Phosflow" 系列產品，提供了相關的抗體、試劑。

Activator (response modifier)	Phosphorylation marker
PMA	ERK 1/2, p38MAPK
hIFN-α	Stat1
hIL-6	Stat3
hIL-2	Stat5
hIL-4	Stat6



T-cell activation profiles monitored with the BD Phosflow T Cell Activation Kit

The BD Phosflow™ Human T Cell Activation Kit is a comprehensive research system that uses flow cytometry to reliably determine the level of key phosphorylated signaling proteins involved in T-cell activation. The histogram overlays to the right show $CD4^+$ and $CD8^+$ T-cell signaling responses to treatment, monitored using the BD Phosflow Human T Cell Activation Kit. Response modifiers are described in the chart above.

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