Unique Markers for the Study of Human Activated Regulatory T Cells: GARP, LAP, and SATB1

Features

- SATB1 is a nuclear protein required for the suppressive function of regulatory T cells (Tregs).[1]
- LAP (latency-associated peptide) forms a complex with TGF-β and is a useful marker for activated Tregs.[2]
- GARP tethers TGF-β to the cell surface of both activated Tregs and platelets.[2]
- Monoclonal antibodies to GARP, LAP, TGF-β, and SATB1 are all available as conjugates and suitable for flow cytometry applications.

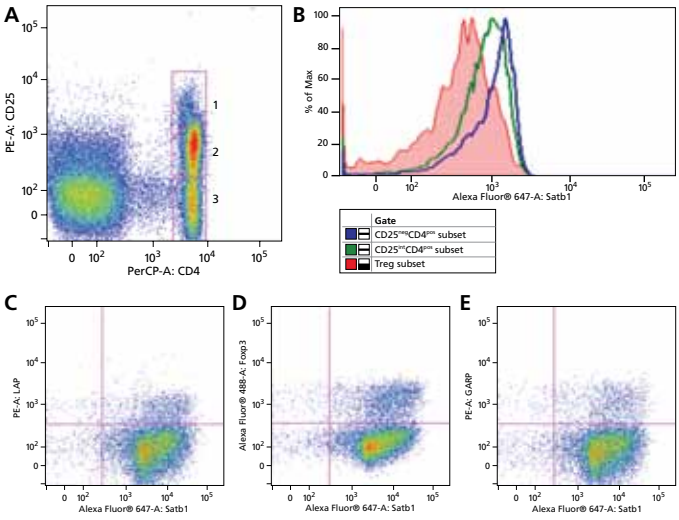

Figure 1. Multicolor flow cytometric analysis of SATB1 expressed in human peripheral blood lymphocytes.

Panels A and B. Human peripheral blood mononuclear cells (PBMCs) were surface stained with PE Mouse Anti-Human CD25 (Cat. No. 555432) and PerCP Mouse Anti-Human CD4 (Cat. No. 347324) antibodies followed by treatment with the Human FoxP3 Buffer Set (Cat. No. 560098) per the recommended protocol. The cells were then stained with Alexa Fluor® 647 Mouse Anti-Human SATB1 (Cat. No. 562378). Events with the forward and side light-scatter characteristics of intact lymphocytes were reanalyzed for CD4 vs CD25 expression (Panel A) to gate for (1) $CD25^{hi}CD4^{+}$ (Treg), (2) $CD25^{int}CD4^{+}$, and (3) $CD25^{-}CD4^{+}$ T-cell subsets as indicated. Panel B shows overlaid SATB1 fluorescence histograms for the three gated $CD4^{+}$ T-cell subsets [ie, CD4 and CD25 gated subsets in Panel 1].

Panels C, D, and E show the expression of SATB1 vs LAP expression (Panel C), SATB1 vs FoxP3 expression (Panel D), and SATB1 vs GARP expression (Panel E) for the stimulated $CD4^{+}$ T-cell populations. PBMCs were stimulated for two days with plate-bound purified NA/LE Mouse Anti-Human CD3 (Cat. No. 555329) and purified NA/LE Mouse Anti-Human CD28 (Cat. No. 555725) and stained with either PE Mouse anti-Human GARP (Cat. No. 562150) or PE Mouse anti-Human LAP (Cat. No. 562260). The cells were then fixed, permeabilized, and stained with Alexa Fluor® 647 Mouse Anti-Human SATB1 (Cat. No. 562378) per the recommended protocol. One set of tubes was stained with Alexa Fluor® 488 Mouse Anti-Human FoxP3 (Cat. No. 560047) and Alexa Fluor® 647 Mouse Anti-Human SATB1 (Cat. No. 562378) following the treatment with Human FoxP3 Buffer per the recommended protocol.

BD continues to support Treg research with high-quality tools to make the most of your precious time and samples. Tregs are a rare cell population representing 5 to 10% of all $CD4^{+}$ T cells. These cells suppress the function of T-effector cells modulating the immune response.

The discovery of the transcription factor FoxP3 as the master regulator of Tregs has led to the discovery of many proteins essential to Treg function and phenotype stability. For example, the role of SATB1 in Treg function was discovered in a study looking at genes repressed by FoxP3.[1] Other proteins such as LAP and GARP can identify subsets of activated Tregs.[2]

SATB1

SATB1 (special AT-rich binding protein-1) binds to a core unwinding element within the matrix attachment DNA region (MAR) and recruits chromatin-modifying factors to the loci of target genes. It is reported to be crucial for T-cell development, and SATB1-null mice die at about 3 weeks of age. T-cell development in these mice appears to be blocked at the $CD4^{+}CD8^{+}$ stage, preventing T-cell maturation.[3]

Many studies have demonstrated plasticity among different populations of T cells. Loss of FoxP3 in Tregs can induce conversion of Tregs into other T-cell types such as Th17 cells.[4] In experiments silencing FoxP3 with small interfering RNA (siRNA), SATB1 expression increased. Upon stimulation, these cells express high levels of T-effector cell cytokines such as interferon-γ and IL-4, indicating that suppression of SATB1 maintains the Treg phenotype.

Clone 14/SATB1 was generated against amino acids 550–667 of human SATB1. This clone is also cross-reactive to mouse and rat SATB1. Applications include Western blot and flow cytometry. Formats include purified and Alexa Fluor® 647 conjugates.

LAP and GARP

TGF-β plays an important role in Treg maintenance and plasticity. TGF-β is synthesized as a pre-pro-form that is processed and modified. Before secretion, the latent or inactive form of TGF-β forms a complex with latency associated peptide (LAP). The proteolytic removal of LAP releases the mature form of the soluble TGF-β dimer.[5] The LAP/TGF-β complex is expressed on activated Tregs and has been reported to be anchored to Treg membranes by GARP (LRRC32) protein.[6,7]

GARP is an 80-kDa transmembrane protein that binds to latent TGF-β. The potential role of GARP in Treg function was discovered in studies looking at changes in mRNA expression following Treg activation. However, the role of this protein in Treg function is unknown.[6,7]

BD has three clones for the study of LAP, TGF-β, and GARP by flow cytometry. Clone 7B11 recognizes human GARP and is available in APC and PE formats. Clone TW4-2F8 recognizes human LAP and is available in the PE format. Clone TW4-9E7 was raised against cells transfected with human TGF-β and is available in the PE format.

Visit **bdbiosciences.com/treg** for more information.

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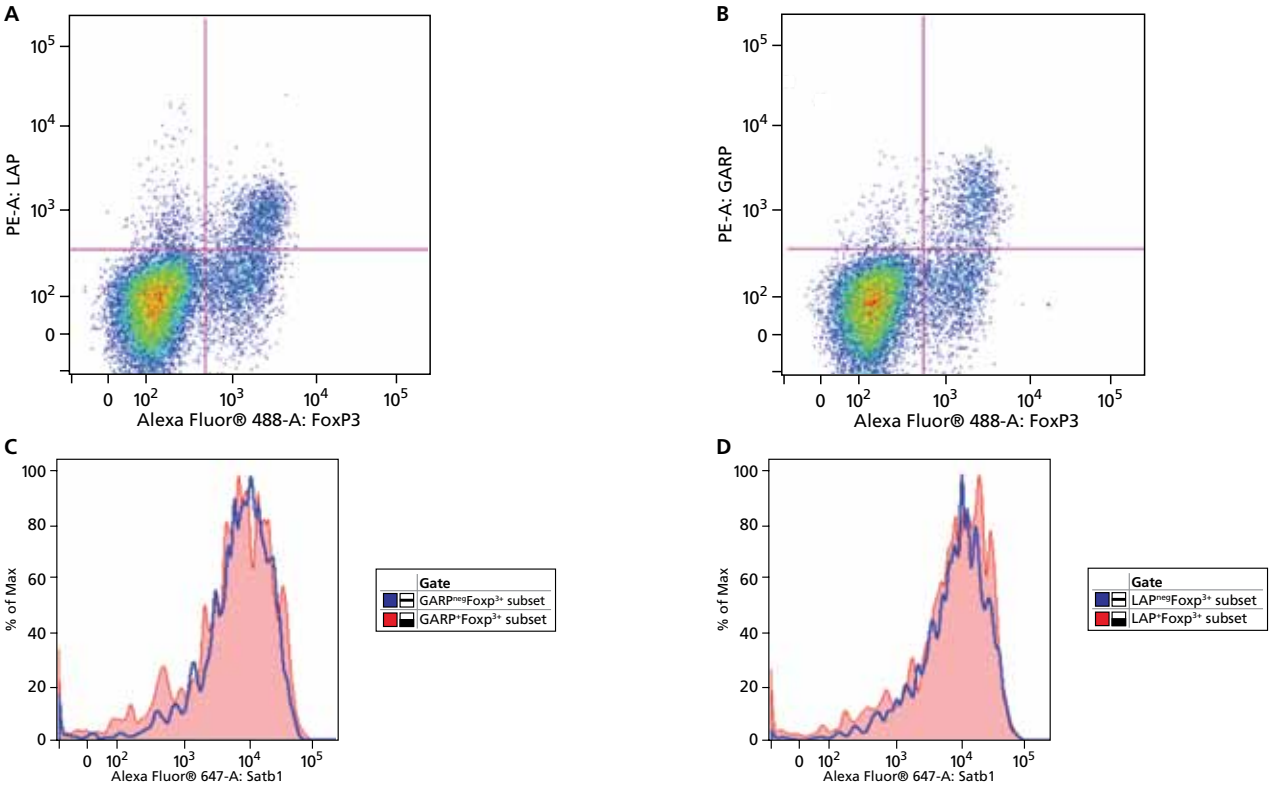

Figure 2. Multicolor flow cytometric analysis of GARP, LAP, and SATB1 expressed on activated human peripheral blood lymphocytes.

Human PBMCs were activated (48 hours) with plate-bound purified NA/LE Mouse Anti-Human CD3 (Cat. No. 555329) and purified NA/LE Mouse Anti-Human CD28 (Cat. No. 555725). The activated PBMCs were washed and stained with either PE Mouse anti-Human GARP (Cat. No. 562150) or PE Mouse anti-Human LAP (Cat. No. 562260). The cells were then fixed, permeabilized, and stained with Alexa Fluor® 488 Mouse Anti-Human FoxP3 (Cat. No. 560047) and Alexa Fluor® 647 Mouse Anti-Human SATB1 (Cat. No. 562378) per the recommended protocol. Two-color flow cytometric dot plots showing the correlated expression patterns of FoxP3 vs LAP (Panel A) or FoxP3 vs GARP (Panel B) were derived from gated events with the forward and side light-scatter characteristics of intact lymphocytes. Panels C and D show the expression of SATB1 on the GARP^{hi}FoxP3^{+}, GARP^{lo}FoxP3^{+} (Panel C histogram overlay) and on the LAP^{hi}FoxP3^{+}, LAP^{lo}FoxP3^{+} (Panel D histogram overlay). Flow cytometry was performed using a BD™ LSR II flow cytometry system.

References

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Ordering Information

Description	Clone	Isotype	Format	Quantity	Cat. No.
Hu GARP	7B11	Ms IgG$_{2b}$, κ	APC	100 Tests	**562341**
			PE	100 Tests	**562150**
Hu LAP	TW4-2F8	Ms IgG$_1$, κ	PE	100 Tests	**562260**
Hu SATB1	14/SATB1	Ms IgG$_1$	Alexa Fluor® 647	100 Tests	**562378**
Hu TGF-β1	TW4-9E7	Ms IgG$_1$, κ	PE	100 Tests	**562339**

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