

Expansion of murine T cells with anti-CD3/CD28 antibodies conjugated to CELLection™ Biotin Binder Dynabeads®

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References

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Introduction

The full complexity of effector (Th1,Th2) and regulatory (Th3, CD4⁺CD25⁺, Tr1) T cell responses and interactions remains a challenging and exciting field of immunology that offers potential for specific therapies particularly in autoimmunity and transplantation. Antigen-specific T cell clones can be isolated and expanded *in vitro* to study their precise phenotype but traditional techniques rely on repeatedly stimulating the clones with irradiated autologous feeder cells which is both time-consuming and a potential contamination source¹. These problems can be circumvented by expanding T cell clones directly with a combination of anti-CD3 and anti-CD28 antibodies. Individual human T cell clones have been successfully expanded with anti-CD3/CD28 antibodies, coupled to magnetic beads, to greater than 10¹⁰ cells with no loss of specificity^{2,3}. Here we describe a quick method to expand spleen or lymph node-derived murine T cells, as well as established T-cell clones, with biotinylated anti-CD3/CD28 antibody conjugated to CELLection™ Biotin Binder Dynabeads®.

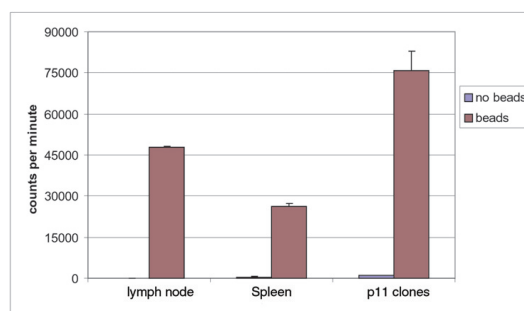


Fig. 1: Anti-CD3/CD28 antibody conjugated to CELLection™ Biotin Binder Dynabeads® promote the expansion of spleen and lymph node-derived lymphocytes and antigen specific CD4⁺ T cell clones.

Methods

Production of murine anti-CD3/CD28 Dynabeads®

Biotinylated antibodies specific for murine CD3 and CD28 (BD Biosciences, UK) were coupled to CELLection™ Biotin Binder Dynabeads® (DynaL Biotech, UK). Beads (500 µl) were aliquoted into a 10 ml tube, and washed three times by adding 1 ml sterile PBS containing 0.1% Tween 20 (PBS-T), placing the tube into a magnetic particle concentrator (MPC®), and aspirating the residue. The beads were resuspended in 500 µl PBS-T, 20 µl (10 µg) of each biotinylated antibody added, and the conjugate placed on a roller for 20 minutes at

room temperature. The ratio of anti-CD3 to anti-CD28 antibody was 1:1. The conjugate was washed a further three times and finally resuspended in 1 ml sterile PBS containing 40% sterile glycerol. The conjugated beads were stored at -20°C until use.

Stimulation of murine T cells and T cell clones.

Spleen or lymph node-derived lymphocytes recovered from BALB/c mice, and T cell clones specific for a collagen-derived peptide, P11, were stimulated with anti-CD3/CD28 conjugated beads. Suspensions of 1 x 10⁶ lymphocytes or 1 x 10⁵ clones in MEMα culture medium (Invitrogen, UK) supplemented with 10% heat-inactivated foetal bovine serum (Invitrogen, UK) were added in 1ml aliquots to 48 well plates. Conjugated beads were added in 2 µl aliquots to each well and the cells incubated at 37°C in 5% CO₂ for up to 4 days. Proliferation was assessed by measuring tritiated Thymidine (Amersham Biosciences Ltd, UK) incorporation over 6 hours.

Quantification of expanded T cell subsets by flow cytometry.

Lymphocytes were washed and resuspended in 100 µl PBS and 2 µl each of antibodies specific for CD3 and CD4 (CD3 ε-PE, CD4-FITC, BD UK), or isotype controls, were added. Cells were incubated for 45 minutes on ice, washed with PBS, resuspended in 300 µl of 1% paraformaldehyde and measured on a FACSCalibur™ (BD Biosciences, UK) flow cytometer.

Results

Biotin binder Dynabeads® conjugated with murine CD3/CD28 antibodies can expand T cells *in vitro*.

Lymphocytes from BALB/c mice or P11 specific T cell clones, when incubated with the Dynabeads® conjugated with anti-CD3/CD28 over a period of three days, were activated and expanded. Fig. 1. demonstrates a comparison of proliferation between cells incubated with or without the beads in the absence of any other stimulus. Spleen and lymph node derived lymphocytes, as well as T cell clones demonstrated clear evidence of proliferation and expansion upon addition of the beads. Within an hour, T cells in contact with beads elongated and transformed to a shape consistent with T cell activation (fig. 2a and 2b). Over a further period of 24-48 hours, activated T cells formed clusters of proliferating cells (fig. 2c). T cells that contacted a bead developed an immunological

synapse contiguous with the bead as demonstrated by the formation of a uropod structure (fig. 2d). Flow cytometry was used to quantify the numbers of CD3⁺ and CD4⁺ lymphocytes before and after expansion for three days with the conjugated beads. The gated lymphocyte population revealed an expansion of the CD3⁺ lymphocyte population from 38.3% to 62% upon incubation with the beads over a period of three days (fig. 3, Row 1a-c). This corresponded to an increase from 3862 to 6206 CD3⁺ lymphocytes whereas cells incubated in the absence of beads decreased to 488 (4.9%) CD3⁺ lymphocytes. Analysis of CD4⁺ expression by these CD3⁺ lymphocytes indicated that numbers of both CD4⁺ and CD4⁻ cells increased upon addition of the beads (fig. 3, Row 2a-c). This implies that the anti-CD3/CD28 beads can expand both CD4 and CD8 murine T cells in much the same way as previously reported for the analogous human T cell expander reagents.

Discussion

We have used CELlection™ Biotin Binder Dynabeads® to prepare a murine T cell expansion reagent analogous to previously described human T cell expansion reagents^{2,3}. The reagent takes less than an hour to prepare but if stored correctly remains stable for several weeks. A further advantage of these beads is that an expanded T cell population can be rested prior to use in T cell assays simply by removing the beads with an MPC. Our protocol for the removal of beads comprises three passes over an MPC to remove the beads, followed by three washes in Hank's Buffered saline solution. This reagent will be particularly useful for the long term culture of murine T cell clones and obviates the requirement for adding autologous feeder cells.

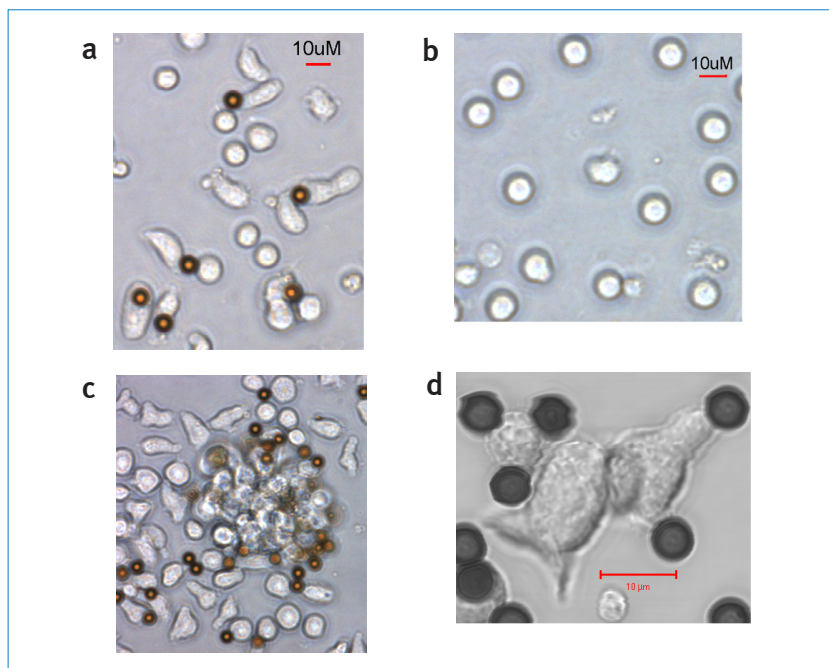


Fig. 2: Expansion of lymphocytes with CELlection™ Biotin Binder Dynabeads®. a and b: cells ligated to beads adopt an elongated morphology characteristic of activated T cells whereas cells deprived of stimulus retain an inactivated phenotype. c: After 24-48 hours cells become clustered around the beads. d: Cells that contact beads respond by forming a uropod structure.

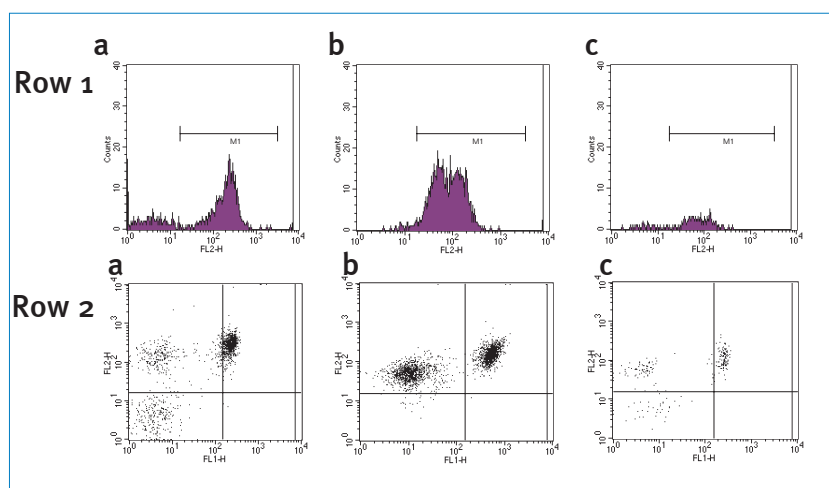


Fig. 3: Incubation with CD3/CD28 CELlection™ Biotin Binder Dynabeads® promotes expansion of CD3⁺ lymphocytes. Row 1 provides an analysis of CD3 cell surface expression (CD3e-PE) while row 2 reveals both CD4⁺ and CD4⁻ lymphocytes are expanded, (CD3e-PE vs. CD4-FITC). a: cells on Day 0; b: Day 3 following addition of beads; and c: unstimulated on Day 3.

Ordering information

Product	Product No.	Content
CELlection™ Biotin Binder Kit	115.21	2 ml kit
CELlection™ Biotin Binder Kit	115.22	10 ml kit
Dynal MPC® -S	120.20	Holds 6 tubes of 20 µl - 2 ml
Dynal MPC® -L	120.21	Holds 6 tubes of 1 - 15 ml