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Induction of Lymphoproliferation by Antigen-Primed Macrophages Across the BD Falcon™ Cell Culture Insert

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Introduction

Cell culture inserts are often used to study the interactions of two cell types without actual physical contact between the two cell populations. However, the pore size of some insert membranes is large enough to allow communication between certain types of cells. The presentation of antigen by macrophages to antigenspecific T lymphocytes, and the subsequent activation of the lymphocytes, requires direct contact between antigen fragments associated with proteins encoded by the major histocompatibility complex of the macrophage and the T cell receptor on the surface of the lymphocyte.¹ Therefore, a coculture system using purified populations of human macrophages and T lymphocytes separated by the cell culture insert was employed to test whether direct cell-cell contact could occur through the 0.45 μm pores of the insert membrane filter.

Materials and Methods

Human Subjects: Peripheral blood mononuclear cells (PBMC) were collected from Bacillus Calmette-Guérin (BCG)-immune and naive volunteers.

Preparation of Cells: Heparinized blood was separated aseptically according to methods adapted from Douvas and Crowle.² PBMC were then resuspended to 0.8×10^7 cells/ml in warm medium with 1% unheated autologous serum and plated onto 0.45 μm BD Falcon Cell Culture Inserts (Cat No. 353090) placed in 6-well plates. Cells were allowed to adhere for one hour at 37°C, 5% CO₂ in a humidified atmosphere after which non-adherent cells (NA) were drawn

off and remaining adherent cells (AD) washed three times. Live, sonicated *Mycobacterium tuberculosis* strain H37Rv organisms were then added at approximately 0.5 bacteria/AD in medium and incubated for 30 minutes, after which time the infection medium was removed, the AD washed to remove extracellular bacteria and a volume of warm medium added. Duplicate cultures were set up in which the AD were not infected. After a second adherence step, the remaining NA were enriched for T lymphocytes using the nylon wool column method of Julius et al.³ The NA T cells were then added to the lower chamber of the 6-well plate at a ratio of five T-enriched cells per AD, separated from the AD by the 0.45 μm pore size insert membrane.

Proliferation Assay: On day seven of co-culture, NA were removed, counted, and placed in 96-well flat-bottom tissue culture plates at 3×10^5 cells/well and incubated with 5 $\mu\text{Ci/ml}$ [3H] thymidine for six hours. Cells were then harvested onto glass wool fiber filters and [3H]-thymidine incorporation into cellular DNA was measured by liquid scintillation spectroscopy.

Phenotypic Analysis: T-enriched cells were removed from co-culture after seven days or taken directly after nylon wool-enrichment and stained using the appropriate monoclonal antibodies for cell surface markers. Fixed and stained cells were then analyzed by flow microfluorimetry.

Scanning Electron Microscopy (SEM): Seven day co-culture cell culture inserts were prepared for SEM according to Technical Bulletin 405. The sputter-coated samples were then scanned on a JEOL JSM-25SII microscope at 15KV.

Results and Discussion

As shown by flow microfluorimetry, the T-enriched cell population routinely consisted of greater than 80% CD3+ (T) cells and an average of 2% contamination with B cells (CD19+) and/or monocytes (CD14+). **Table 1** shows results of the T-cell proliferation assay in which cells from all six donors, whether BCG-immune or naive, exhibited responses ranging from 4 to 300 times that observed in uninfected (control) cultures. The reactivity of BCG-naive cells to *M. tuberculosis*-infected macrophages may indicate T-cell recognition of ubiquitous, cross-reactive heat shockproteins⁴ or may reflect an underlying cellular response to mycobacterial antigens which was not elicited by the tuberculin skin test used to screen the volunteers.

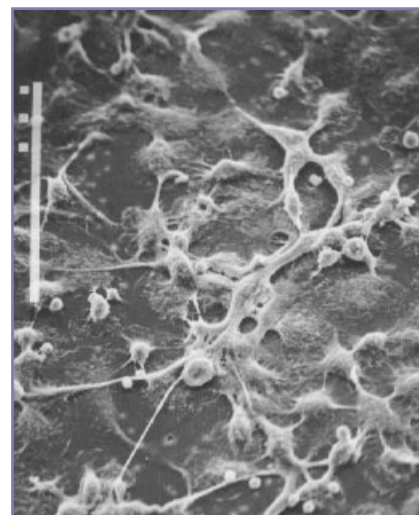


Figure 1: SEM of *Mycobacterium tuberculosis*-infected adherent cell culture growing near confluence on cell culture insert. Bar measures 100 μm .

Figures 1-3 are representative of the AD population growing on the cell culture membrane. Membrane pores are visible but it is obvious that the cells are also spread over and covering pores. Macrophages averaged 12 μm in diameter and larger, while filopodia were in the range of 0.2 μm in thickness. Figure 4 demonstrates the opposing side of the cell culture insert membrane with AD cell membrane extruding through the insert membrane pores.

The electron micrographs corroborate the *in vitro* blastogenesis data and suggest that macrophages and lymphocytes, separated by the cell culture insert membrane, can make sufficient contact through the membrane pores to result in activation of T-cells.

Table 1: Proliferation of T-enriched cells separated from macrophages by the cell culture insert.

Donor	[3H]-thymidine incorporated (mean \pm sd) by T-enriched cells in coculture with:		% Contamination with:		
	M. tb. H37Rv-infected AD	Uninfected AD	CD14+	CD19+	
BCGnaive	1	23,097 \pm 1,380	183 \pm 58	2.7	2.5
	2	10,390 \pm 1,479	301 \pm 109	0.9	2.4
	3	6,517 \pm 552	435 \pm 176	1.9	4.5
BCGimmune	1	22,496 \pm 866	75 \pm 12	1.9	1.8
	2	1,753 \pm 757	239 \pm 68	0.2	1.0
	3	1,583 \pm 155	439 \pm 197	0.8	3.2

*All cpm values are the means of triplicate determinations.

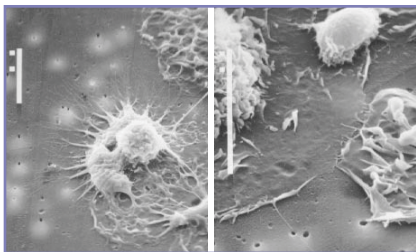


Figure 2a (left) and 2b (right): SEMs of AD attached to culture insert membrane; membrane pores (\pm 0.45 μm) are visible as are long, narrow cellular filopodia. Bar measures 10 μm .

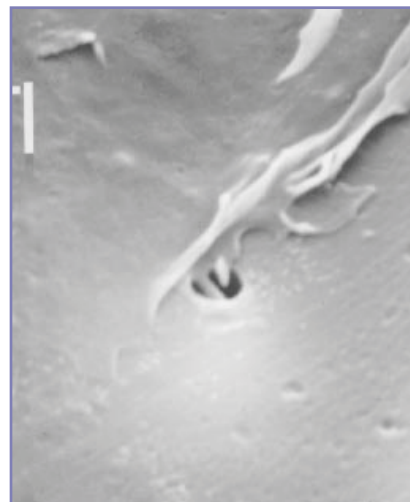


Figure 3: High magnification (15,000x) of AD membrane extending through a membrane pore. Bar measures 1 μm .

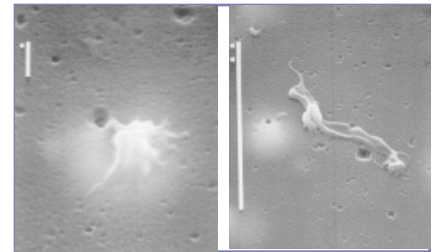


Figure 4a (left) and 4b (right): SEMs of underside of insert with AD membrane extruding through insert membrane pores.

References

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