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Relative Efficiency of Human Langerhans' Cells and Blood Derived Dendritic Cells as Antigen-Presenting Cells

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T_4 cells recognize antigens together with HLA class II molecules in the membrane of antigen-presenting cells (APC). The magnitude of the induced T cell response is in part dependent upon the APC's amount of MHC-class II molecules. Langerhans' dendritic cells (LC) express 50–100 times more HLA-DR molecules than monocytes (Mo) and blood derived dendritic cells (DC). We report here that LC are more efficient APC than DC from the same donor, indicating that the APC capacity of dendritic cells isolated from different organs is correlated to their expression of HLA class II gene products. (Received March 2, 1985.)

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Dendritic cells are present in most organs (1, 2). Only dendritic cells of the epidermis, spleen and blood have been extensively studied. These cells are shown to be potent antigen-presenting cells (APC), but no comparison of the APC-function of different kinds of dendritic cells from the same individual have previously been performed.

Among normal epidermal cells (EC), LC is the only accessory cell (3, 4) and constitutes 3–5% of the EC. LC are potent stimulators of allogeneic lymphocytes and also induce strong antigen activation of T cells (5, 6). The antigen-presenting cell of human blood was previously believed to be monocytes (Mo), but recent investigations indicate that blood derived dendritic cells (DC) are the main accessory cells (7, 8, 9). We have previously shown (5, 7) that T cell proliferation induced by antigen swas much higher when DC and LC were used as APC compared with Mo and adherent cells (Mo+DC) respectively. Furthermore we have shown that LC express quantitatively more HLA-DR than Mo (10), while Mo and DC express similar amounts of HLA-class II molecules (8).

Phenotypic differences have made it possible to develop procedures for enrichment of epidermal dendritic cells (Langerhans' cells) (6, 11) and blood derived dendritic cells (DC) (8). We here compare the antigen-presenting capability of LC and DC from the same donor.

MATERIAL AND METHODS

Cell donors

The cell donors were unrelated in-patients with various skin diseases at the Department of Dermatology.

Antigen preparation

Purified protein derivative of tuberculin (PPD) solution in phosphate buffer (2 mg/ml) without preservative (obtained from the Veterinary Institute, University of Oslo) was freshly diluted in RPMI 1640 medium supplemented with penicillin and streptomycin and 20% normal human serum to a final concentration of 5 μg/ml.

Preparation of EC and enriched LC

Epidermis was separated from dermis at the basement membrane in vivo by means of a suction blister device (12). The sheets of epidermis obtained were then dissociated by means of trypsin, washed and suspended in medium RPMI 1640 with L-glutamin (Gibco-Bio-Cult, Glasgow, Scotland), supplemented with penicillin, streptomycin and 20% normal human serum. Viability was usually more than 90% and the cell suspensions contained 2–6% DR positive cells. No monocytes/macrophages were detected in the epidermal cell suspensions, determined by a Mab specific for monocytes (1D5) by an indirect immunofluorescence technique. LC were then separated from the majority of EC using a rosette forming technique as previously described (6). In short, LC were sensitized with the monoclonal antibody OKT6. This suspension was then mixed with ox red blood cells (ORBC) coated with rabbit anti mouse IgG antiserum. Within one hour at 37°C rosettes were formed and separated from the EC by Percoll flotation. The OKT6 LC were recovered from the pellet after haemolysis of the ORBC. The mean percentage of rosette-forming OKT6 LC in the pellet was 69%±16.

T cell preparation

Peripheral blood mononuclear cells (PBM) were first prepared by flotation on Lymphoprep. T-lymphocyte-enriched suspensions were then prepared by rosetting with sheep cells treated with aminoethylisothiuronium bromide hydrobromide (AET) (Sigma Chemical Co., St. Louis, Mo., USA) as described earlier (4). The non-T cell fraction was used to prepare monocytes and dendritic cells.

Monocytes and dendritic cells

The non-T cell fraction was placed on petridishes coated with gelatin and fibronectin (8). Dendritic cells (DC) and Mo attached to the fibronectin within 1–2 hours when incubated at 37°C. The B cells were then decanted and the plates washed several times with RPMI 1640 with L-glutamin (Gibco Bio-Cult, Glasgow, Scotland) supplemented with penicillin and streptomycin. The petridishes with Mo and DC were then covered with 10 ml of RPMI supplemented with 20% normal human serum. After 24 hours of incubation at 37°C the DC detached from the fibronectin and could be obtained from the supernatant. Contaminating Mo among the DC were then removed by incubating the cells on Petri dishes coated with IgG for 15–30 min at 37°C. The Mo adhere via their Fc receptors to the IgG, and purified DC remain in the supernatants. Monocytes were eluted from the fibronectin dishes after 24 hours by adding EDTA for 5–10 min at 37°C. These Mo were further purified by being poured on to IgG plates. Contaminating cells (e.g. DC, B-cells) are then decanted, the plates washed several times before the Mo are detached by vigorous pippeting after addition of EDTA supplemented with 1% IgG. The Mo population was more than 95% pure determined by Mo specific Mab (1D5). Purified DC contained less than 20% contaminating cells as judged by reactivity with Mab's specific for other mononuclear cells.

Cell culture technique

5×10^4T lymphocytes were cocultured with or without PPD and with various numbers of autologous APC such as enriched LC or DC in the wells of round bottomed microtitre plates (Model 15-MRC 96, Linbro Scientific, Inc., Hamden, Conn., USA) in a humid 5% CO_2 atmosphere at 37°C for seven days. Each well contained 170 µl Medium RPMI-1640 supplemented with penicillin, streptomycin and 20% normal human serum. 1 µCi (^{3}H)-thymidine was added 18 h before harvesting, which was performed with a semi-automatic multiple cell culture harvester (Skatron, Lierbyen, Norway). Incorporation of (^{3}H)-thymidine was measured by means of a liquid scintillation counter and expressed as mean of triplicate cultures ± SE.

RESULTS AND DISCUSSION

To compare the antigen-presenting capacity of Langerhans' cells (LC) and blood derived dendritic cells (DC) from the same donor, 5×10^5 T cells were mixed with various numbers of autologous DC or LC in the presence or absence of PPD. The results from one typical experiment are shown in Fig. 1, and a summary of ten experiments is shown in Fig. 2. It can be seen that LC on an average are approximately 3–4 times more potent APC than DC. The response induced by 25000 unfractionated epidermal cells (EC) is shown for comparison, and is similar to that of 1000 purified LC. EC contain 3–4% LC (OKT6$^+$ cells; 13, 14). Thus, a LC content of 4% in 25000 EC may explain the APC activity observed with unfractionated EC. In most of the experiments MO did not induce any response at all, and

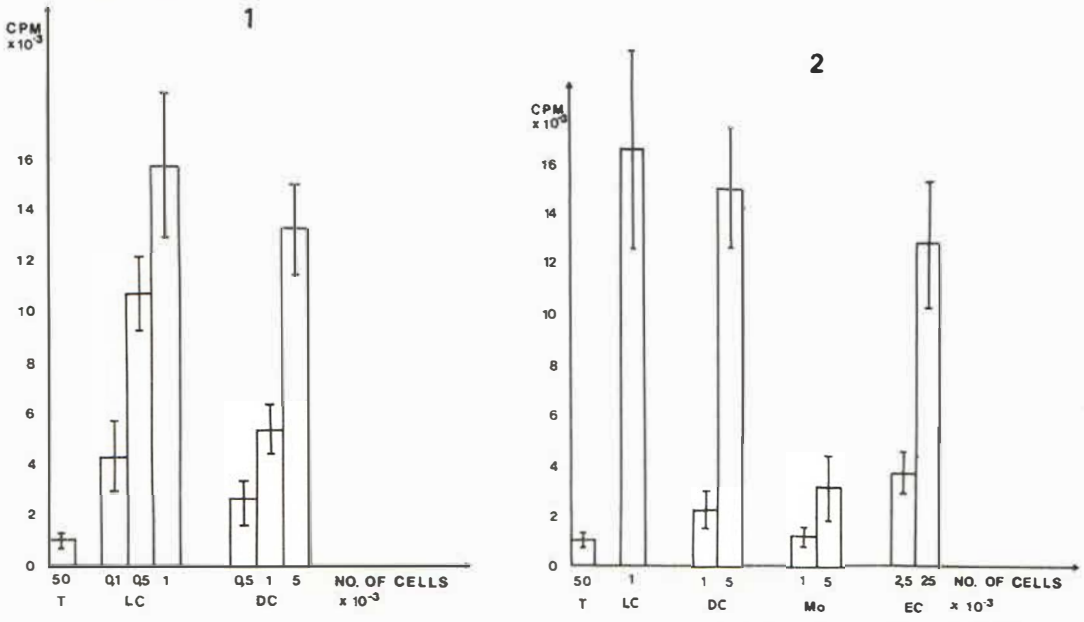

Fig. 1. The T cell proliferative response towards PPD when Langerhans' cells (LC) and dendritic cells (DC) are used as antigen-presenting cells. Values are cpm ± SE.

Fig. 2. The T cell proliferative response towards PPD when Langerhans' cells (LC), blood derived dendritic cells (DC), monocytes (Mo) and epidermal cells (EC) are used as antigen-presenting cells. Values are mean of 10 experiments ± SE.

the weak T cell proliferation by Mo in some of the experiments may have been caused by a few contaminating DC. Mo without DC seem to suppress rather than induce a proliferative response to soluble antigens (7) in vitro.

T_4 cells recognize antigens together with HLA class II molecules in the membrane of APC. Recently it was shown that the T cell response was the same as long as the product of the antigen and MHC-class II moleules on the APC membrane was constant (15). These findings demonstrate the importance of the concentration of HLA class II molecules on the APC for induction of T cell responses. We have recently shown that LC express 50–100 times more HLA-DR than Mo and DC (10). Our observations that Langerhans' cells are more efficient APC than blood derived DC may reflect this difference in the quantity of HLA class II gene products expressed by the two cell types.

A second signal involved in APC function is IL-1. The enriched LC populations were contaminated by 10–30% Keratinocytes (K). Both LC (16) and K (17) are able to produce IL-1 providing optimal conditions for T4 cell activation. On the other hand, production of IL-1 by the DC has so far not been reported. In our DC preparation the contaminating Mo (8%) and B cells (12%) might however, have produced some IL-1.

Dendritic cells, although not yet precisely defined, are a family of large cells having a profusion of cell processes. They comprise Langerhans' cells (18), indeterminate cells (19), interdigitating cells (20, 21), veiled cells (22), follicular dendritic cells (23, 24), interstitial dendritic cells (1, 25) and blood derived dendritic cells (2, 7, 8). Judged from the dendritic cells most extensively studied (LC, blood- and spleen-derived dendritic cells) it seems that the different kinds of dendritic cells have similar functions, i.e. they are important accessory cells in the induction of immune responses. A prerequisite for APC

function is expression of class II gene products, and all DC so far studied express class II molecules in the membrane.

Our findings indicate that the APC function of dendritic cells "subsets" is correlated to the expression of class II gene products. The interrelationship between blood DC and LC is unknown, and the two cell types express no common phenotypic markers (8), except from HLA class II gene products. It is thus unclear whether LC is a more mature DC that have differentiated from a blood borne precursor such as the blood DC—in analogy to Mo being precursor for tissue macrophages.

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