

## Letter to the Editor

### EXPRESSION OF MHC AND ADHESION/COSTIMULATION MOLECULES OF DENDRITIC CELLS FROM HUMAN BLOOD DURING THEIR DIFFERENTIATION IN VITRO

Dear Editor:

Since their discovery more than 25 years ago, dendritic cells (DCs) have emerged as the most potent member of the class of antigen-presenting cells (APC). A number of experimental studies have established the key role played by DCs in the immune system, and provide a rationale for using DCs as natural adjuvants for cancer immunotherapy. Because of their capacity to stimulate T lymphocytes, particularly naive T cells, DCs have been proposed as the basis for vaccines designed for the treatment of cancer (Caux et al., 1991; Steinman, 1991; Young and Inaba, 1996; Nair et al., 1997). DCs can be stimulated by different cytokines, particularly granulocyte/macrophage-colony stimulating factor (GM-CSF), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), or interleukin-4 (IL-4), to develop into active DCs over 1 wk in culture. Recently, however, several studies have shown that the combination of GM-CSF and IL-4 generates significantly larger numbers of DCs from monocytes/macrophages (MO/M) (Steinbach et al., 1995; Romani et al., 1996).

In this study, CD14<sup>+</sup>-MHC Class I and II were identified as cells adherent to plastic at 2 h, but after 1 wk of culture with GM-CSF and IL-4, the cells were morphologically and phenotypically changed. It was clear that CD14<sup>+</sup> cells underwent differentiation in vitro and assumed a dendritic morphology.

Since the efficiency of cytotoxic T-cells (CTL) priming by MO has been shown to be weaker than that of DCs and dependent upon the level of expression of MHC and adhesion/costimulation molecules, and since either M or DCs differentiate from MO, depending on culture conditions (i.e., GM-CSF versus GM-CSF/IL-4), we decided to investigate the differences in the kinetics of immunological expression of several surface antigens and adhesion/costimulation molecules during the differentiation of DCs or M from blood MO.

Briefly, 42 ml of peripheral blood from five healthy volunteers were centrifuged on Ficoll-Hypaque (Sigma Scientific, USA) to obtain peripheral blood mononuclear cells (PBMC). These cells were then plated ( $1 \times 10^6/3$  ml per well) into six-well culture plates (Costar, Cambridge, MA) in AIM-V (GIBCO-BRL, Grand Island, NY). After 2 h at 37° C, nonadherent cells were removed, whereas the adherent cells were cultured at 37° C in a humidified 5% CO<sub>2</sub>/95% air incubator, in medium supplemented with recombinant human GM-CSF [800 U/ml], Immunex, Seattle, WA and IL-4 [1000 U/ml], Genzyme, Cambridge, MA) or GM-CSF alone at the same concentration. Every 2 d, 1 ml of spent medium was replaced by 1.5 ml of fresh medium containing 1600 U/ml GM-CSF and 1000 U/ml IL-4, to yield final concentrations of 800 and 500 U/ml, respectively. In the matched cultures from the same donor, only GM-CSF was used at the same concentration. Cells were evaluated for surface marker expression using FACS analysis and a panel of monoclonal antibodies (mAbs) at different time points (i.e., time 0 [cells remaining at 2 h after rinse], days 3, 5, 7, and 10) (Table I).

An average of three experiments were performed for each donor and FACS analysis was evaluated using matched control cultures tested at the same time.

To visualize their morphology DCs were cytocentrifuged for 4 min at 400 rpm on a microscope slide using a Cytospin-2 centrifuge (Shandon, Southern Products, Astmoor, UK). Slides were then fixed in methanol/acetone, stained with May-Grunwald-Giemsa solution and analyzed by light microscopy on a Zeiss Axiophot microscope (Zeiss, Germany).

Collectively, our data suggest that DCs progenitors were identified as CD14<sup>+</sup>/MHC Class II<sup>+</sup> cells adherent to plastic, negative for other lineage specific markers. After 1 wk in culture with GM-CSF/IL-4, the percentage of cells were evaluated for DCs dimension. Their immunophenotype was evaluated by FACS (i.e., cells strongly expressing HLA-DR<sup>+</sup>, CD86<sup>+</sup>, CD40<sup>+</sup>, and CD14<sup>+</sup>); they ranged from 85 to 95% of the total cell population. These data indicate that under appropriate culture conditions, MO differentiate directly into DCs.

The purity of the populations can be assessed by three approaches with comparable results. One approach uses mAb and flow cytometry (Steinman, 1991) to phenotype the cell fractions. Markers that DCs lack are CD3 (T-cells), CD14 (MO), CD19/20 (B-cells), and CD56/57 (NK cells). The second approach is to observe the cell fractions live by video microscopy. The third approach is to test MLR stimulatory function.

The large CD14<sup>+</sup>CD1a<sup>-</sup> cells, which corresponded to MO as confirmed by cytology and cytochemistry (data not shown), differentiated into CD1a<sup>+</sup> DCs to the extent as unseparated cells when cultured with GM-CSF/IL-4.

MHC Class I molecules were highly expressed on DCs cultured in GM-CSF/IL-4. In contrast, MHC Class II antigens were always significantly upregulated on DCs when compared to M, and this difference in expression progressively increased until day 10. These cells displayed a significantly higher expression of MHC Class II than the adherent population of M. The fact that in cultures with GM-CSF/IL-4 the striking majority of MO differentiated into DCs, while in the absence of IL-4 only a few precursors showed enough flexibility in changing to a phenotype resembling DCs, supports the hypothesis of a common precursor.

Two new markers, CD83 and p55, recently have been shown to be expressed by the small subset of mature dendritic cells in cultured human blood (Zhou and Tedder, 1996). In this regard, previous reports have shown that CD83 is a maturation marker of blood DCs. No single marker uniquely delineates human DCs. CD1a has been considered a hallmark DCs marker, and therefore was investigated in our study. FACS analysis showed a low percentage of cells expressing CD1a during the culture period. The importance of the expression of CD1a on cells expressing DCs morphology has

TABLE 1

EXPRESSION OF MHC AND ADHESION/COSTIMULATION MOLECULES OF DENDRITIC CELLS DURING THEIR DIFFERENTIATION IN VITRO<sup>a</sup>

mAbs	Day 0	Day 3	Day 5	Day 7	Day 10
<i>Cdla</i> <sup>+</sup>					
HLA-DR	++/-	+++/-	+++/-	+++/-	++/-
HLA-ABC	++/-	+++/-	+++/-	+++/-	+++/-
ICAM-1	-/-	+++/-	+++/-	+++/-	++/-
CD80(B7-1)	-/-	+/-	++/-	++/-	+++/-
CD86(B7-2)	++/-	+++/-	++/-	++/-	++/-
LFA-3	+++/-	+++/-	+++/-	+++/-	+++/-
CD14	+++/-	++/-	-/-	-/-	-/-

<sup>a</sup> PBMC were cultured for 10 d in the presence of IL-4/GM-CSF. Data represent MFI and are indicated as follows: — no detectable staining, MFI 10<sup>0</sup>-10<sup>1</sup> corresponding to isotype-matched nonreactive control mAbs. Each + corresponds to an increase in mean fluorescence intensity of one logarithm: +, MFI 10<sup>1</sup>-10<sup>2</sup>; ++, MFI 10<sup>2</sup>-10<sup>3</sup>; +++, MFI 10<sup>3</sup>-10<sup>4</sup>. The results are representative of three experiments.

been recently challenged by the findings that CD1a-positive and CD1a-negative DCs displayed similar capacity in stimulating human naive T cells. Therefore, as previously suggested, DCs morphology is not necessarily coupled to CD1a expression or to DCs function.

CD40 ligand (CD40L), the ligand for CD40 on APC, is essential for the initiation of antigen-specific T-cell responses. The most important interactions are between CD40 and CD154, and B7 and its ligands Cd28 and CTLA-4. Several functional studies have shown that CD40 maturation signals are critical for the direction of cellular immune responses in tumor immunity. CD40 expression has been shown to be crucial not only for B cell growth, isotype switching, and Ig synthesis, but also for optimal T cell priming. Recent reports have shown that CD40 stimulation on APC is linked to the upregulation of CD86 molecules and an increase in T cell stimulatory activity (Van Gool et al., 1996).

Our data showed a dramatic upregulation of CD40 on DCs compared to M. Therefore, CD40 stood out as a marker, significantly and consistently expressed on DCs at all stages of their maturation, and presumably is an important component in their phenotype. Surface expression of ICAM-1 and LFA-3 molecules is involved in antigen-independent conjugate formation and has been shown to be particularly important when the affinity of the T-cell receptor (TCR) for antigen MHC expressed on APC is low. ICAM-1 expression was low in freshly isolated precursors. At day 3, both cultures showed a significant increase in the expression of this marker, with M expressing significantly more ICAM-1 than DCs. However, while M's expression peaked at this time and downregulated ICAM-1 to undetectable levels thereafter, DCs progressively increased their expression until day 10. Therefore, we believe that, in agreement with previous reports, the transient induction of ICAM-1 on M may represent the consequence of a nonspecific stimulation, likely related to plastic adherence or to other unidentified stimuli. In contrast, the kinetics of expression of ICAM-1 on DCs support a pathway of expression linked to a programmed maturation process, having the ultimate purpose of increasing accessory function. Finally, the kinetics of LFA-3 expression was in dramatic contrast to that of all the other antigens studied. Indeed, expression of LFA-3 was detectable on M at all time points tested, and such expression was always significantly higher than the levels expressed on DCs. This

suggests that the LFA-3 pathway of costimulation does not likely play a key role in differentiating the activation of T cells by DCs versus M.

We conclude, that our data indicate significant differences in surface antigen expression between DCs versus M, as these cells differentiate from blood MO. Specifically, such differences appeared major for MHC Class II, CD86, CD40, and ICAM-1. In contrast, DCs showed a progressive increase of expression of all antigens studied throughout the culture period. The enhanced accessory ability of DCs compared to M in naive T cell priming may be related to qualitative and quantitative differences in the expression of these immunologically important surface molecules. These qualitative and quantitative immunological differences with respect to other cells, such as M, enable their different proprieties in naive T-cell priming. These results support the concept that modification by cytokines may be useful for directly activating T-cells for cancer immunotherapy.

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Maurizio Chiriva-Internati  
Fabio Grizzi<sup>1</sup>  
Ombretta Orbetegli

Seah Lim  
Paul L. Hermonat  
Nicola Dioguardi

Center for Immunology & Microbial Disease  
Albany Medical College  
Albany, New York (M. C.-I., S. L.)  
Scientific Direction (F. G., N. D.)  
and Research Laboratory (O. O.)  
Istituto Clinico Humanitas  
Rozzano, Milan  
Italy

Fondazione Michele Rodriguez  
Istituto Scientifico per le Misure Quantitative in Medicina  
Milan, Italy (F. G., N. D.)  
Department of Obstetrics and Gynecology  
Division of Gynecologic Oncology  
University of Arkansas  
Little Rock, Arkansas (P. L. H.)

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<sup>1</sup>To whom correspondence should be addressed at Scientific Direction,  
Istituto Clinico Humanitas, Via Manzoni 56-20089 Rozzano, Milano, Italy.  
Fax: 39-0282-244590; E-mail: fabio.grizzi@humanitas.it