All-trans-retinoic acid upregulates CD1a on human monocyte-derived dendritic cells (MoDC): implications for autologous melanoma-specific tumor vaccination

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Introduction

Retinoic acid (RA) acts on both early as well as late stages of cellular immunity in that it regulates the differentiation of myeloid bone-marrow precursors and thymocytes, and as well modulates the activity of skin-resident immune cells.

Therapeutically, topical RA has been proven benefical for the treatment of myeloid and skin-associated tumors. We thus hypothesized that RA might modulate the function of myeloid lineage-derived cells of the skin, such as epidermal LANGERHANS cells (LC) which may be of major importance in the control of developing skin tumors.

Because LC are regional representatives of the ubiquitous dendritic-cell system [1], we here cultured monocyte-derived dendritic cells (MoDC) [2,3] to investigate whether RA regulates the expression of LC surface molecules that are required for the efficient presentation of protein or lipid antigens.

This pilot study should give a first clue on whether the demonstrable tumor-protective effect of RA might be due to the immuno-modulation of LC.

Materials and Methods

Monocytes were enriched from buffy coat leukocytes by density gradient centrifugation and 1-h adherence at 37°C. For MoDC differentiation, 5 x 10^6 monocytes per Petriperm dish (Bachofer) were cultured in serum-free CG medium (Vitromex) plus 20 IU GM-CSF, 10 IU IL-4, and 10 IU IFN-γ per ml (Genzyme). On day 3, half of the cultures (n = 3) received 0.2 µg/ml of the hydrophilic RA derivative, all-*trans*-RA (Sigma). All cultures were terminated on day 6.

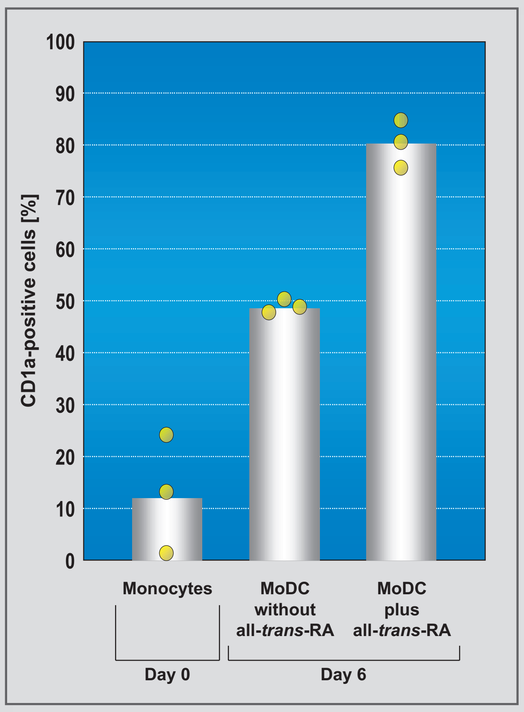
On days 0 and 6, monocytes or MoDC were stained directly for CD1a (FITC-conjugated OKT6 IgG1; Ortho) and HLA-DR, -DP, and -DQ (PE-conjugated CR3/43 IgG1; Dako). Cells expressing these markers were detected by flow cytometry with the FACStar^{PLUS} IV (Becton-Dickinson), gated against FITC- or PE-conjugated IgG1 isotype controls (Dianova) and are here given as percent positive cells (single values and arithmetic means).

Results

On day 0, 12.96% (1.44-24.20%) of the monocyte starting population were positive for CD1a. In contrast, 48.65% (47.20-50.03%) of the MoDC generated by GM-CSF, IL-4, and IFN-γ expressed this surface glycoprotein after 6 days of culture. We have earlier shown that IFN-γ leads to the secretion of TNF-α by developing MoDC [4], and it is known that GM-CSF and TNF-α cooperate in the generation of CD1a$^+$ LC [5]. The expression of CD1a in such MoDC cultures is, therefore, easily explained.

However, when differentiated in the presence of all-*trans*-RA, the percentage of CD1a$^+$ MoDC increased to 80.15% (76.13-84.08%). It is established knowledge that the cytoplasmic RA derivative, retinyl phosphate, serves as a carrier for oligosaccharide residues and, thereby, participates in the synthesis of glycoproteins [6]. We therefore suggest that RA may directly contribute to the upregulated synthesis of the glycoprotein CD1a. As a result, when comparing the yields of 48.65% *vs.* 80.15% CD1a$^+$ cells, addition of all-*trans*-RA led to an increase in CD1a-expressing MoDC by 64.75%.

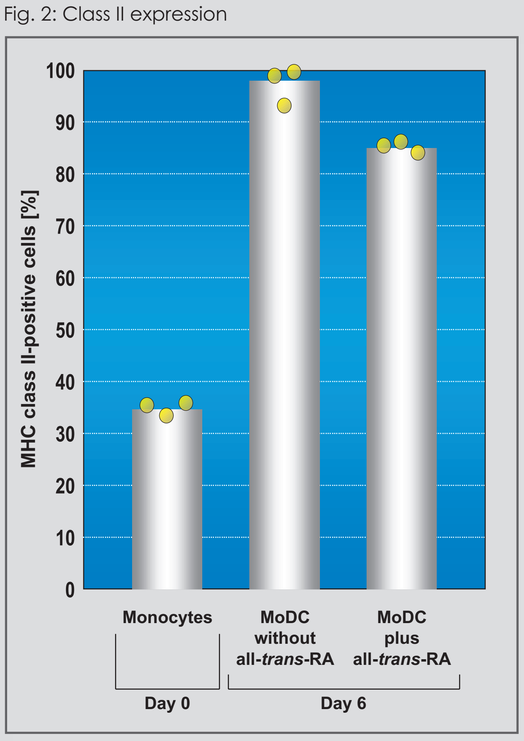
Fig. 1: CD1a expression



While 34.65% (32.50-36.04%) of freshly isolated monocytes expressed HLA-DR-, DP, and -DQ, the percentages of cells revealing MHC class II rose throughout the course of MoDC differentiation. However, whereas 97.15% (93.24-99.82%) of the control MoDC carried MHC class II molecules, only 84.99% (83.21-86.33%) of the cells generated in presence of all-*trans*-RA were positive for HLA-DR,-DP, and -DQ.

This finding is in contrast to studies with isolated LC where all-*trans*-RA upregulated HLA-DR (as well as CD1c, CD11c and the cells´ antigen-presenting capacity) [7]. Although further clarifying studies are needed, this inconsistency may well be due to the differing functional stages of LC and MoDC, and to the diverse culture conditions.

Fig. 2: Class II expression



Discussion

Retinoids generally appear to support the performance of LC. That is, apart from the findings with RA by Meunier *et al.* [7] and ourselves, Halliday and co-workers showed that the RA aldehyde, retinal, prevents the reduction in the density of LC after ultraviolet irradiation [8]. It therefore appears that the polyisoprenoid compounds retinoic acid, retinal, and retinol may all be important modulators of LC function.

In this pilot study, all-*trans*-RA surprisingly appeared to act slightly suppressive on the expression of MHC class II. Yet, because class II was only determined on the sixth day of culture, it now has to be addressed whether all-*trans*-RA may possibly alter the time kinetic of class II expression by developing MoDC.

More importantly, however, we found an all-*trans*-RA-dependent increase in the percentage of LC-like CD1a$^+$ MoDC by almost 65% under the specific conditions employed herein. To our knowledge, this finding for the first time demonstrates a positive effect of all-*trans*-RA on the expression of this important antigen-presenting molecule.

Provided this in-vitro effect may be transferred to conditions in the skin, topical (all-*trans*-)RA might either upregulate the expression of CD1a by epidermal LC, increase the rate of LC differentiaton from intraepidermal monocyte precursors, or even both these alternatives.

A suggestion for the anti-tumor effect of topical RA
Current knowledge implies that all molecules encoded by the CD1 gene family present lipid antigens [9,10]. Thus, as to the increased generation of CD1a$^+$ cells by all-*trans*-RA and to the beneficial effect of RA in the treatment of skin tumors, CD1a might be important in the presentation of hitherto unidentified lipophilic tumor antigens. Indeed, inhibitory cytokines such as IL-10 that are released by tumor cells [11] or UV-irradiated keratinocytes [12] may impair the antigen-presenting capacity of neighboring LC [13] and lead to tumor tolerance [14]. We suggest that suppressive cytokines downregulate the expression of CD1 molecules by LC and, thus, affect the presentation of lipophilic tumor antigens. This defect is possibly corrected by the topical application of RA.

A suggestion for improved cellular tumor vaccines
Vaccination with autologous MoDC is among the most promising novel strategies to eradicate malignant tumors. Preliminary clinical trials are currently underway [e.g., 15-18]. However, because the dendritic cells presently employed still appear prototypic, we suggest that further studies are needed to determine whether the supplementation of state-of-the-art MoDC protocols with additional regulator molecules might increase the efficacy of these vaccines. Specifically, selected vitamin-A derivatives could help to custom-tailor autologous LC-like vaccines with increased potency for the successful treatment of malignant melanoma.

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