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LETTERS TO THE EDITOR

Dendritic Cell Differentiation

Antonio Curti, Elisa Ferri, Simona Pandolfi, Alessandro Isidori, and Roberto M. Lemoli

Institute of Hematology and Medical Oncology "L. & A. Seràgnoli"
University of Bologna
Bologna, Italy

Dauer et al. (1) presented a method to differentiate $CD14^+$ cells into mature dendritic cells (DC) within 48 h (FastDC). FastDC displayed a DC-like morphology, down-regulated CD14, and induced proliferation of autologous T cells against soluble Ags as efficiently as standard monocyte-derived DC (Mo-DC). Given the implications of these data for the generation of DC as anti-tumor vaccine, we tried to confirm the results. Monocytes were purified with MACS CD14 isolation kit (Miltenyi Biotec, Germany) and were cultured (1×10^6 cells/ml) for 24 h in complete medium supplemented with GM-CSF (50 ng/ml; Schering Plough, Kenilworth, NJ) and IL-4 (800 IU/ml; Endogen, Woburn, MA). Then, cells were cultured for an additional 24 h with the following maturation stimuli: TNF-α (25 ng/ml; CellGenix, Freiburg, Germany), IL-1β (10 ng/ml; CellGenix), IL-6 (1000 IU/ml; CellGenix), and PGE_2 (1 μg/ml; CellGenix). The methods used are the same as those used by the authors. Standard mature Mo-DC were generated from monocytes as previously described (2, 3). In our hands, monocytes cultured for 48 h in presence of the above cytokines displayed a monocytic morphology and expressed CD14, CD86, and HLA-DR. As compared with standard mature Mo-DC, FastDC showed a blunted capacity to induce proliferation of allogeneic T cells and to activate KLH-specific proliferative response of autologous T cells. In conclusion, our results indicate FastDC as activated monocytes. These cells are likely to be good APCs but do not fit the main features of mature DC. Their use as anti-tumor vaccine should be further investigated.

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The Authors Respond

We have recently shown that dendritic cells (DCs) generated from monocytes by a novel 2-day strategy possess equal capacity to induce Ag-specific Th1-type immune responses compared with standard monocyte-derived DCs (1). Curti et al. now report that monocytes treated with this 2-day protocol maintained their original phenotype and failed to induce allogeneic or KLH-specific autologous T cell proliferation. Specifically, they observed maintained expression of CD14 and lack of CD83 expression in monocytes that were cultured for 24 h with GM-CSF and IL-4 followed by 24-h stimulation with a combination of proinflammatory mediators (TNF-α, IL-1β, IL-6, and PGE_2). We have shown that

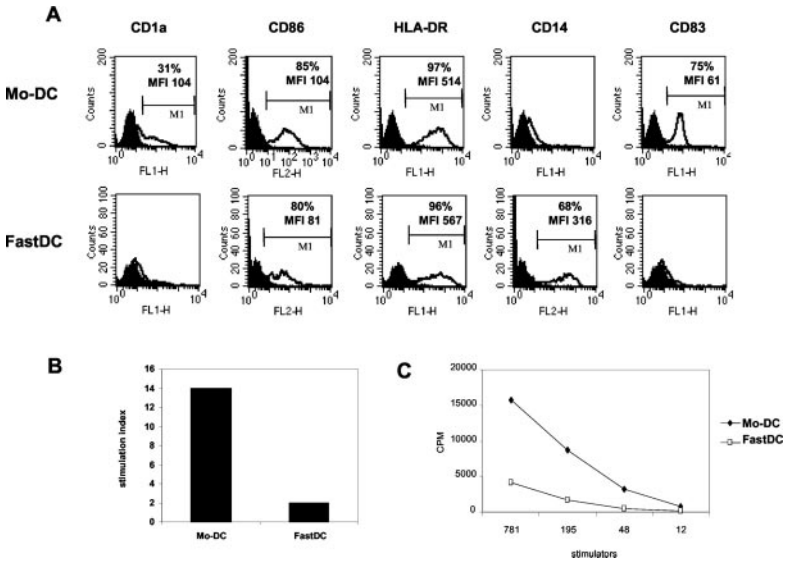

FIGURE 1. *A*, The phenotypic profile of Mo-DC and FastDC at the end of culture. Overlay diagrams show the expression of the relevant Ags vs isotype-matched Ab (negative controls). The figure shows both the percentage of positive cells and the mean fluorescence intensity (MFI) value. The results are representative of three independent experiments. *B*, T cells (1×10^5) were incubated with 198 irradiated DC in presence of 50 μg/ml KLH. Autologous DC alone represented the negative control and were used to calculate the stimulation index as follows: SI = cpm of T cell responders plus KLH-pulsed stimulators KLH, divided by cpm of T cell responders plus stimulators. The results are representative of three independent experiments. *C*, Increasing numbers of irradiated DC were used to stimulate 1×10^5 allogeneic mononuclear cells. The results are representative of three independent experiments.

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monocytes isolated from PBMC either by plastic adherence or CD14 positive selection using MACS display an immature DC immunophenotype after 24 h of culture with GM-CSF and IL-4 (1). Unfortunately, Curti et al. do not provide any information on the immunophenotype observed in their cultures after the first 24 h. Our own unpublished observations indicate that the 2-day differentiation and activation protocol fails to yield fully mature DCs if CD14 expression is preserved after the first 24 h of the culture process. In this scenario, proinflammatory activation will not induce maturation of DCs but rather an activated monocytic phenotype as described by Curti et al. Prolonged CD14 expression may be due to early activation of monocytes during the isolation process. Curti et al. also state that *Fast*DC may only represent activated monocytes and thus have a blunted capacity to stimulate proliferative T cell responses. In fact, we have already demonstrated that *Fast*DC display a fully mature DC phenotype, express equal levels of costimulatory molecules as well as MHC molecules and secrete equal levels of IL-12 compared with standard monocyte-derived DCs (1). If phenotypically mature DCs are generated, these cells also possess equal capacity to induce Ag-specific autologous T cell proliferation (1). Consistent with these results, we were able to show that our 2-day protocol does not necessarily require the use of proinflammatory mediators, but also yields fully mature DCs if other stimuli known to activate standard monocyte-derived DCs are used; ATP—which has been identified previously by our group as a potent activator of standard monocyte-derived DCs (2)—also induces maturation of *Fast*DC. The same synergistic effect of ATP and TNF that has been observed in activation of standard monocyte-derived DCs can be seen in *Fast*DC (Fig. 1*A*). In contrast to the results presented by Curti et al., *Fast*DC are also potent stimulators of allogeneic T cell proliferation in our hands (Fig. 1*B*). Moreover, if *Fast*DC are extensively washed after maturation is completed and subsequently cultured in medium alone without addition of any cytokines or growth factors, they maintain their mature DC phenotype for at least 48 h, indicating that these cells represent terminally differentiated DCs rather than activated monocytes (Fig. 1*C*). However, we agree with Curti et al. that further studies are required to test whether *Fast*DC loaded with tumor antigen can be used for anti-tumor vaccination.

Marc Dauer, Katharina Schad, Jan Herten, Stefan Endres, and Andreas Eigler

Division of Clinical Pharmacology and Section of Gastroenterology
Medizinische Klinik Innenstadt
University of Munich
Munich, Germany

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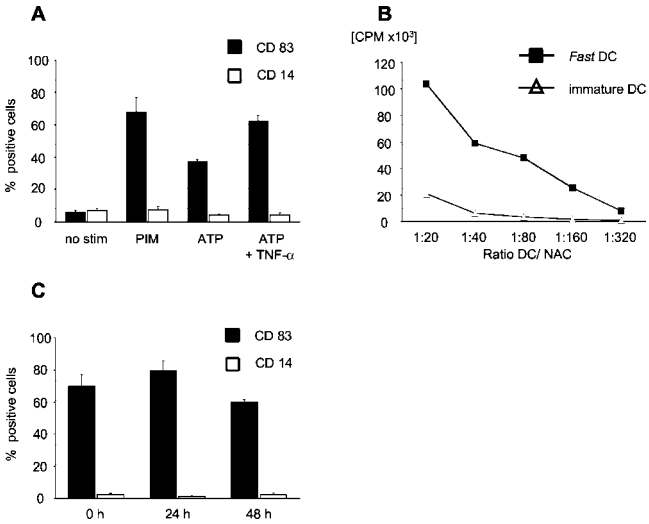

FIGURE 1. *A*, ATP and TNF-α synergistically induce maturation of *Fast*DC. Monocytes were either cultured for 48 h with GM-CSF (1000 U/ml) and IL-4 (500 U/ml) alone or additionally stimulated with proinflammatory mediators (PIM: 10 ng/ml IL-1β, 1000 U/ml TNF-α, 1 μM PGE_2), ATP (100 μM) or ATP plus TNF-α for the last 24 h. Expression of CD83 and CD14 was determined by FACS analysis. Data represent means ± SEM of four experiments with different donors. *B*, *Fast*DC are potent stimulators of allogeneic T cell proliferation. *Fast*DC or immature DCs were cocultured with 2×10^5/200 μl allogeneic nonadherent PBMC (NAC) in different ratios. On day 5 of coculture, proliferation was determined by standard [^{3}H]thymidine incorporation assay. One representative experiment of three is shown. *C*, *Fast*DC are terminally differentiated. *Fast*DC were extensively washed and resuspended in medium without addition of cytokines or growth factors. Immediately after completion of the 48-h culture period (0 h) and at different time points of the wash-out phase, CD83 and CD14 expression was determined by FACS analysis. Data represent means ± SEM of three experiments with different donors.

Signaling and Cell Surface Expression of μH Chains in the Absence of Light Chains

Schuh and colleagues (1) recently reported that a transgenic Sp6-μHC can be transported to the cell surface in the absence of surrogate and conventional light chains (rag-deficient background). They ascribe this property to the Sp6-variable region, and speculate that the production of some μ chains with particular variable regions could explain the "leaky" phenotype of λ5-deficient mice. We have found that a transgenic Sp6-human μ heavy chain was inefficient in correcting pre-B cell development in rag-proficient, λ5-deficient mice, while a V-less human μ chain relieved the requirement for surrogate light chains (2). Our data suggest that there is nothing particular with the Sp6 variable region and that the good efficiency in promoting pre-B cell development in the rag-deficient background might be related to homeostatic compensation due to Ig and B cell deficiency. Another interpretation of their data, that we favor, is that any rearranged μ gene can give rise to a minority of VDJ-less transcripts by splicing of the leader exon to the CH1 exon, which, in mouse as in man, can induce V-less protein production (3, 4). This is consistent with the low expression of μ chain on the surface. In the transgenic model de-

scribed by Schuh and colleagues, the presence or the absence of variable region on the B cell surface should be tested.

Daniel Corcos

Hôpital Cochin
Paris, France

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The Authors Respond: Signaling and Cell Surface Expression of μH Chains in the Absence of Surrogate and Conventional Light Chains

In his comments about our manuscript entitled "Cutting Edge: Signaling and Cell Surface Expression of a μH Chain (μHC) in the Absence of λ5: A Paradigm Revisited" (1), Corcos argues that the positive effect of the transgenic Sp6-μHC on the progression of cells from the pro-B to the pre-B cell stage in the absence of λ5 might rather be the result of signals delivered by a truncated μHC lacking the V_H region (V-less μHC) than by the wild-type Sp6-μHC.

The idea that truncated V_H-less μHCs promote the transition of cells from the pro-B to the pre-B stage even in the absence of a complete surrogate light chain (SLC) has been verified in transgenic mouse models by Corcos and coworkers (2) as well as Schlissel and coworkers (3). However, in our system we can exclude the presence of truncated V_H-less μHCs, since, as already published in an earlier manuscript by Hess et al. (see Fig. 2*D* in Ref. 4), we detected in bone marrow B-lymphoid cells by Western blot analysis only a signal corresponding to a 70-kDa full-length μHC but never a signal indicating the presence of a shorter chain. Therefore, developmental progression of pro-B cells in the absence of λ5 is driven in our transgenic mouse model by a full-length wild-type Sp6-μHC rather than a V_H-less μHC.

In his comments, Corcos also mentioned that a chimeric μHC composed of the mouse V_HSp6 region and the human Cμ region (chimeric mouse/human Sp6-μHC) was inefficient in promoting pre-B cell development in λ5-deficient, Rag-proficient mice (2). This statement is somewhat misleading, since the contour blots in Fig. 4 in Ref. 2 clearly show that the chimeric mouse/human Sp6-μHC supports the progression of pro-B cells into CD43-negative pre-B cells in transgenic λ5-deficient mice when compared with nontransgenic λ5-deficient mice, albeit with a lower efficiency than in λ5-proficient transgenic mice. Therefore, the findings by Corcos et al. (2) are in line with our findings and support the conclusion that a full-length Sp6-μHC promotes progression of pro-B cells in the absence of a λ5 chain, although with a lower frequency than in the presence of λ5.

We also think that the positive effect of a transgenic Sp6-μHC on pro-B cell progression in the absence of λ5 is due to a structural feature of the V_HSp6 region, since Nussenzweig and coworkers found that a chimeric mouse/human μHC using another murine V_H region (i.e., V_H3–38; Ref. 5) does not support the progression of pro-B cells in a transgenic 3-38-μHC, λ5/Rag-double-deficient mouse (6).

In summary, our findings (1) as well as that of Corcos et al. (2) and Nussenzweig et al. (6), strongly support the idea that the leaky phenotype of B cell maturation in λ5-deficient mice could be explained by the production of some μHCs that gain in a V_H-dependent manner surface transport-and signaling competency even in the absence of a complete SLC.

Hans-Martin Jäck, Wolfgang Schuh, Silke Meister, and Edith Roth

Division of Molecular Immunology
Department of Internal Medicine III
Nikolaus-Fiebiger Center
University of Erlangen
Erlangen, Germany

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