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*J Immunol* 1999; 163:57-61; ;
http://www.jimmunol.org/content/163/1/57

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*The Journal of Immunology* is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852
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Print ISSN: 0022-1767 Online ISSN: 1550-6606.
Polyriboinosinic Polyribocytidylic Acid (Poly(I:C)) Induces Stable Maturation of Functionally Active Human Dendritic Cells

Rob M. Verdijk, Tuna Mutis, Ben Esendam, Janine Kamp, Cees J. M. Melief, Anneke Brand, and Els Goulmy

For vaccination strategies and adoptive immunotherapy purposes, immature dendritic cells (DC) can be generated from adherent monocytes using GM-CSF and IL-4. Presently, the only clinically applicable method to induce stable maturation of DC is the use of supernatants of activated monocytes (monocyte-conditioned medium (MCM)). MCM contains an undefined mixture of cytokines and is difficult to standardize. Here we report that stable maturation of DC can be simply induced by the addition of polyriboinosinic polyribocytidylic acid (poly(I:C)), a synthetic dsRNA clinically applied as an immunomodulator. Poly(I:C)-treated DC show a mature phenotype with high expression levels of HLA-DR, CD86, and the DC maturation marker CD83. This mature phenotype is retained for 48 h after cytokine withdrawal. In contrast to untreated DC, poly(I:C)-treated DC down-regulate pinocytosis, produce high levels of IL-12 and low levels of IL-10, induce strong T cell proliferation in a primary allo MLR, and effectively present peptide Ags to HLA class I-restricted CTL. In conclusion, we present a simple methodology for the preparation of clinically applicable mature DC. The Journal of Immunology, 1999, 163: 57–61.

Dendritic cells (DC) are professional APC (1). Immature DC effectively capture and process native protein Ags (2). Mature DC express high levels of MHC and co-stimulatory molecules and effectively initiate primary T cell responses (3). Recently, methods to generate large numbers of DC from adherent monocytes have been developed (4). These DC generated with GM-CSF and IL-4 display an immature phenotype unless treated with additional factors. Treatment of immature DC with single cytokines or combinations of cytokines such as TNF-α, IFN-β, IFN-γ, IL-1β, or PGE₂ induces reversible maturation of DC (4–6). Treatment with supernatants of activated monocytes (monocyte-conditioned medium (MCM)) induces stable maturation of DC (7). It is, however, difficult to generate standardized batches of MCM for clinical purposes.

Viral infection and inflammatory products such as LPS, bacterial DNA, and CpG-oligodeoxynucleotides trigger the maturation of DC (8–10). A synthetic dsRNA, polyriboinosinic polyribocytidylic acid (poly(I:C)), is often used in models of viral infection. Poly(I:C) is a potent IFN inducer (11, 12) and can activate monocytes to produce CSF (11), IL-1β (11), IL-12 (12), and PGE₂ (11).

Poly(I:C) has been used in various clinical trials with little or no toxicity (13, 14).

Here we describe that poly(I:C) can induce stable maturation of DC. We demonstrate that poly(I:C)-treated DC are comparable to DC treated with MCM in both their phenotypical and functional characteristics. This simple method can easily be used in closed culture systems for the generation of clinical applicable mature DC.

Materials and Methods

Culture medium

Culture medium consisted of RPMI supplemented with 100 U/ml penicillin 100 μg/ml streptomycin (both from Life Technologies, Paisley, U.K.), and 2% heat-inactivated autologous heparinized plasma or pooled human serum.

Reagents

Staphylococcus aureus Cowan I strain Pansorbin cells (SACS) were purchased from Calbiochem (La Jolla, CA). Poly(I:C) was purchased from Sigma (St. Louis, MO; lot 27H4009) and dissolved in PBS, and aliquots of 1 mg/ml were preserved at −20°C. The minor histocompatibility Ag (mHag) HA-1 peptide (15) was synthesized using a semiautomatic multiple peptide synthesizer; its purity was checked by reverse phase HPLC.

PBMC and T cell clones

PBMC were isolated by Ficoll-Hypaque density gradient separation of blood collected from healthy blood donors. Naive cord blood cells were isolated by Ficoll-Hypaque density gradient separation of cord blood. The mHag HA-1-specific HLA-A*0201-restricted CTL clone (16) was used in proliferation experiments.

Preparation of MCM

PBMC, mixed from five randomly selected healthy blood donors were allowed to adhere to petri dishes (Falcon, Lincoln Park, NJ) for 2 h at 37°C (50 × 10⁶/petri dish). Nonadherent cells were removed with forceful pipetting. The adherent fraction was cultured in 8 ml of culture medium/petri dish supplemented with either SACS (1/10,000) or 50 μg/ml of poly(I:C). After 24 h the cell-free supernatants were filtered (0.22 μm pore size) and stored at −20°C.
DC culture

DC were cultured from adherent PBMC in the presence of 800 U/ml GM-CSF and 500 U/ml IL-4 as described by Romani et al. (7). On day 7, 50% MCM or different concentrations of poly(I:C) (range, 1–50 μg/ml) were added to induce DC maturation. The MCM- or poly(I:C)-treated DC as well as untreated DC were cultured for another 72 h. For determination of phenotype stability, DC were washed three times and cultured for an additional 48 h in culture medium without cytokines, MCM, or poly(I:C).

Phenotype analysis

DC were labeled using FITC- or PE-labeled specific mAb to CD14, CD40, CD80, CD86, CD83, and HLA-DR (all from Becton Dickinson, Mountain View, CA). The fluorescence intensity was measured using the FACScan (Becton Dickinson).

Pinocytosis assay

DC treated with SACS-MCM and poly(I:C)-treated and untreated DC were harvested and stored on ice in culture medium for at least 30 min before initiation of the assay. FITC-conjugated BSA (BSA-FITC, Sigma) was added at a final concentration of 1 ng/ml, and DC were incubated for 1 h at 37°C or on ice (background staining). Cells were washed twice in ice-cold medium and fixated with PBS containing 1% paraformaldehyde before FACS analysis.

Cytokine measurements

Adherent PBMC were cultured for 7 days with GM-CSF and IL-4. Cell-free culture supernatants were harvested after 24, 48, or 72 h of further culture of the DC at a concentration of 5 × 10^7/ml with or without poly(I:C) (12.5 μg/ml). IL-12 p40 (R & D Systems, Abingdon, U.K.), IL-12 p70 (Diaclone, Besancon, France), and IL-10 (Schering Plough, Dardilly, France) release in the supernatants was measured by specific sandwich ELISA.

Primary MLR

Serial dilutions (1.5 × 10^4 to 3 cells/well) of irradiated (30 Gy) stimulator cells were cultured in triplicate with 1.5 × 10^5 responder cells in 96-well round-bottom plates (Costar, Corning NY). After 96 h 1 μCi/well [3H]thymidine (New England Nuclear, Boston MA) was added to the wells. The cells were harvested after 16 h, and [3H]thymidine incorporation was determined by liquid scintillation counting. The results are expressed as the mean of triplicate cultures. The SEM of the results never exceeded 15%. As stimulator cells, autologous PBMC or random CBC were used. As stimulator cells, PBMC, monocytes, immature DC, and DC treated with SACS-MCM or with poly(I:C) were used.

T cell proliferation assays

The HA-1-specific CTL clone was used as responder cells (1.5 × 10^4 cells/well) and was cocultured with irradiated (30 Gy) stimulator cells (10^3 cells/well) in 96-well round-bottom microtiter plates for 72 h. The mHag HA-1 peptide was added in the assay in a serial dilution of 1 ng/ml to 10 μg/ml. Sixteen hours before harvesting, the cells were labeled with 1 μCi/well [3H]thymidine. [3H]thymidine incorporation was determined by liquid scintillation counting. The results are expressed as the mean of triplicate cultures. The SEM of the results never exceeded 15%. As stimulator cells, monocytes, immature DC, and DC treated with SACS-MCM or with poly(I:C) were used.

Results

Poly(I:C) induces mature surface phenotype of DC

We have tested whether supernatants of poly(I:C)-activated monocytes (pIC-MCM) or purified poly(I:C) alone can induce DC maturation. Untreated DC and DC treated with SACS-MCM were used as controls. Treatment of immature DC for 72 h with SACS-MCM, pIC-MCM, or purified poly(I:C) resulted in the typical DC

![FIGURE 1. Poly(I:C)-treated DC display typical dendritic morphology.]

DC culture from adherent monocytes with GM-CSF and IL-4 for 7 days followed by treatment with purified poly(I:C) at a concentration of 12.5 μg/ml for 3 days. Photomicrographs were taken on day 10 of culture with medium resolution (×100). The cells clearly show a typical dendritic morphology.

![FIGURE 2. Poly(I:C) induces mature surface phenotype of DC.]

DC were cultured from adherent monocytes with GM-CSF and IL-4 for 7 days followed by treatment with SACS-MCM, poly(I:C)-MCM, poly(I:C) (12.5 μg/ml), or no treatment for 72 h. Filled histograms depict specific Ab staining; open histograms depict controls. DC treated with poly(I:C)-MCM, poly(I:C), or SACS-MCM express high levels of HLA DR, CD86, and the mature DC marker CD83. Untreated DC express these cell surface markers at significantly lower levels. As expected, immature DC show a very low expression of CD83. The results in this figure are representative of three independent experiments.
morphology (Fig. 1) and induced significant maturation, as reflected by up-regulation of HLA-DR, CD86, and CD83 (Fig. 2). As expected, expression of the maturation marker CD83 was low or absent on untreated DC, and CD14 expression was low or absent in all DC preparations. In subsequent experiments, the maturational effect of poly(I:C)-MCM could be almost completely abrogated by RNase treatment. This demonstrates that dsRNA actually is the main active component of the MCM (data not shown). Treatment of DC with 12.5 μg/ml poly(I:C) for 72 h appeared optimal. Poly(I:C) concentrations over 25 μg/ml were toxic, whereas concentrations of poly(I:C) lower than 1 μg/ml did not induce any maturation (data not shown). Based on these results, we decided to use only purified poly(I:C) in a concentration of 12.5 μg/ml for maturation induction of the DC cultures.

Poly(I:C)-treated DC remain stable in phenotype after cytokine withdrawal

We investigated whether DC treated with poly(I:C) retain their mature phenotype following cytokine withdrawal for 24 and 48 h. DC treated with poly(I:C) retained stable high expression of HLA-DR, CD86, CD80, and CD40 with no expression of CD14 when cultured without IL-4 and GM-CSF for 48 h. These results were comparable to those obtained with SACS-MCM-treated DC (data not shown). Beyond 48 h of culture without cytokines the number of mature DC decreased rapidly.

Poly(I:C)-treated DC down-regulate pinocytic activity

Mature DC lose their pinocytic activity (17, 18). To evaluate whether the poly(I:C)-induced phenotypic maturation of DC was accompanied by down-regulation of Ag uptake, we measured BSA-FITC uptake by untreated DC, SACS-MCM-treated DC, and poly(I:C)-treated DC. In contrast to untreated DC, BSA-FITC uptake was markedly down-regulated in poly(I:C)-treated DC, comparable to that in DC cultured with SACS-MCM (Fig. 4). The uptake of BSA-FITC is a metabolically active process; metabolically inactive cells, incubated on ice, did not take up BSA-FITC (Fig. 4, open histograms).

Poly(I:C)-treated DC produce high levels of IL-12 and low levels of IL-10

Mature DC can produce both IL-12 and IL-10 depending on the maturation stimulus. We tested IL-12 and IL-10 production by DC upon poly(I:C) treatment. No poly(I:C) treatment resulted in the absence of IL-12 (Fig. 5A) or IL-10 (Fig. 5B) production. Indeed, poly(I:C)-treated DC produce a high level of IL-12 p40 and p70. IL-12 secretion increased until 48 h and remained stable after 72 h. Poly(I:C)-treated DC did not produce significant amounts of IL-10 (Fig. 5B). The SACS-MCM contained high amounts of IL-10 (data not shown).

Poly(I:C)-treated DC are efficient APC in primary allo mixed lymphocyte reactions

The Ag-presenting capacity of poly(I:C)-treated DC to naive cord blood responder T cells was assayed in a primary allo MLR. The
stimulatory properties of poly(I:C)-treated DC were compared with those of PBL, monocytes, immature DC, and SACS-MCM-treated DC. As depicted in Fig. 6A, poly(I:C)- or SACS-MCM-treated DC induced comparable allo mixed lymphocyte reactions that were most markedly seen with 15,000 DC/well, in a 1:10 stimulator:responder ratio. Immature DC and monocytes were clearly inferior in stimulating capacity at all stimulator:responder ratios. Thus, poly(I:C) treatment induced DC that were potent inducers of primary allo T cell responses. The stimulation of low, but significant, T cell proliferation in an auto mixed lymphocyte reaction is a property described only for mature DC (19). Indeed, DC treated either with poly(I:C) or with SACS-MCM, but not monocytes or immature DC, were able to induce a strong auto mixed lymphocyte reaction (Fig. 6B).

Poly(I:C)-treated DC effectively present peptide to a MHC class I-restricted mHag HA-1-specific CTL clone

The potential of poly(I:C)-treated DC to present peptide Ags to CTL was evaluated using the mHag HA-1 peptide. HA-1 peptide-pulsed DC from an HLA-A*0201-positive, HA-1-negative individual were analyzed with the HA-1-specific T cell clone. Peptide-pulsed poly(I:C)-treated DC are most efficient in the induction of proliferation of mHag HA-1-specific HLA-A*0201-restricted CTL clone (Fig. 6C).

Discussion

The effects of poly(I:C) on the maturation of immature DC were investigated and compared with the effects of SACS-MCM, which is known to induce maturation of DC. Our results demonstrate that treatment of immature DC with poly(I:C) results in DC with stable and high expression of the costimulatory molecules, CD80 and CD86, and expression of the maturation marker CD83. Accordingly, the phenotypic maturation is accompanied by down-regulation of pinocytic activity and production of high levels of IL-12 and neglectable amounts of IL-10. Moreover, poly(I:C)-treated DC are potent stimuliators of primary allo mixed lymphocyte reactions and adequately present peptide Ags to HLA class I-restricted mHag-specific CTL.

The stability of the mature form of DC is crucial for in vivo application of Ag-pulsed DC for immunotherapy protocols. We show that poly(I:C)-treated DC retain their stable phenotype at least 48 h after withdrawal of cytokines.

The exact mechanism by which poly(I:C) induces maturation changes is unknown. Poly(I:C) has been described to bind to scavenger receptors of macrophages (20, 21). It is not known whether this binding is accompanied by internalization or even if internalization is required for activation. Poly(I:C)-MCM can be expected to contain residual amounts of poly(I:C). RNase treatment of poly(I:C)-MCM almost completely abrogated the maturation effect. This indicates that indirect effects, such as the induction of a complex mixture of autocrine or paracrine factors, are not sufficient for maturation induction and that the presence of poly(I:C) in the DC culture is required. These findings are consistent with observations that treatment of immature DC with single cytokines or combinations of cytokines, such as TNF-α, IFN-β, IFN-γ, IL-1β,
or PGE₂ induces partial and reversible maturation of DC (4–6). Induction of the production of IFN, IL-1, and nitric oxide in both human endothelial cells and murine macrophages by treatment with poly(I:C) has been shown to be regulated via activation of NF-κB (22, 23). It is therefore likely that the poly(I:C) treatment of DC may result in activation of the NF-κB pathway.

Depending on the factors used for the maturation, the cytokine profile of DC will be determined (18). IL-12 is an important initiator of the Th1-like T cell responses (24) involved in antiviral and antitumor immunity. IL-10 is generally regarded as a Th2-type cytokine. IL-10-producing DC have been described to be driven T cells in the direction of a Th2-like immune response (25). Poly(I:C)-treated DC produce high levels of IL-12 and very low levels of IL-10, which is most relevant for the immunotherapy protocols.

The potential of clinical applicable DC in anticancer therapy has been emphasized (26). DC have been used with success in antitumor lymphoma therapy (27). We have recently developed protocols for the ex vivo generation of cytotoxic T cell lines for adoptive immunotherapies. This simple method can easily be adapted to closed culture systems for the generation of large amounts of clinically applicable mature DC.

Note added in proof. While this paper was reviewed, Cella et al. (30) reported data confirming the data described in this paper.

Acknowledgements

We thank Jaqueline v. Beckhoven for kindly providing cord bloods, Delphine Rea for helpful discussion and performing the IL-12 p70 ELISA, and Frits Koning for critical review of the manuscript.

References


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