The Journal of Immunology

Kinetics of Antigen-Induced Phenotypic and Functional Maturation of Human Monocyte-Derived Dendritic Cells

Szu-Min Hsieh, Sung-Ching Pan, Chien-Ching Hung, Hsing-Chun Tsai, Mao-Yuan Chen, Chun-Nan Lee and Shan-Chwen Chang

J Immunol 2001; 167:6286-6291; ;
doi: 10.4049/jimmunol.167.11.6286
http://www.jimmunol.org/content/167/11/6286

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Kinetics of Antigen-Induced Phenotypic and Functional Maturation of Human Monocyte-Derived Dendritic Cells[1]

Szu-Min Hsieh,[2,3]* Sung-Ching Pan,[2]* Chien-Ching Hung,[2†] Hsing-Chun Tsai,* Mao-Yuan Chen,* Chun-Nan Lee,[‡] Shan-Chwen Chang*

Dendritic cells (DCs), a critical component of innate immunity, are the most potent APCs. When DCs mature, they can elicit strong T cell responses. We studied the kinetics of Ag-induced phenotypic and functional maturation of human monocyte-derived DCs using an in vitro T cell-independent culture system. With this model, we herein show that an Ag that has recently or repetitively been exposed ("exposed Ag") rapidly induces a high level of maturation; however, an Ag that has never or only remotely been exposed ("unexposed Ag") slowly induces a low level of maturation. The kinetics of Ag-induced maturation of DCs possibly implies a novel mechanism for immunological memory that would provide maximal host protection from repetitively invading pathogens in the environment. *The Journal of Immunology,* **2001, 167: 6286–6291.**

Protective immunity results from the cooperation of Ag-nonspecific innate immunity and Ag-specific adaptive immunity (1, 2). Dendritic cells (DCs) [4] comprise a family of potent APCs and play critical roles in host defense (3, 4). When DCs mature, they not only link innate immunity and adaptive immunity by presenting the processed Ag and costimulatory signals to activate specific T cells (5–8) but also play an instructive role in adaptive immunity by regulating Th1-Th2 development (9–12). Therefore, mature DCs are essential for the development of protective T cell immunity in vivo and have been widely used to elicit T cell effector functions in vitro (13).

In a previous study (14), we found that under Ag stimulation, the duration of DC culture required to elicit detectable CTL activity varied among different volunteer donors. The required duration of culture seemed to be related to whether the volunteers had prior exposure to an Ag. We surmised that if the host had been exposed to the Ag, the Ag-induced maturation and the immunostimulatory capacity of DCs might be enhanced, and adaptive immunity might be elicited earlier and more effectively than if the host had never exposed. This mechanism, if it exists, may defend the host more effectively against invasion by pathogens that are frequently encountered in the environment, therefore increasing the chances of survival of the host.

Materials and Methods

Monocyte isolation

All donors who provided peripheral blood were healthy volunteers, and none had HIV infection or had received any antiretroviral therapy. Human PBMCs were prepared from heparinized blood and isolated by differential centrifugation over endotoxin-free Ficoll-Paque (Pharmacia Biotech, Wikstromes, Sweden). Monocytes were negatively isolated from PBMC using the immunomagnetic beads coated with mAbs against human CD2, CD7, CD16, CD19, and CD56 (Monocyte Negative Isolation kit; Dynal, Oslo, Norway) according to the instructions of the manufacturer.

To enhance the efficiency of lymphocyte depletion, beads coated with anti-human CD3 mAb (Dynal) were added to these isolated cells largely according to the instructions of the manufacturer. The procedure was modified by raising the ratio of beads to cells to >40:1, keeping the final concentration of beads at $>2 \times 10^7$/ml, and extending the incubation period (at 4°C) to at least 30 min with gentle, continuous rotation.

Monocyte culture

Negatively isolated monocytes were suspended in complete RPMI (Life Technologies, Gaithersburg, MD) supplemented with penicillin/streptomycin, 10% FCS (Life Technologies), recombinant human IL-4 (1000 U/ml; BD PharMingen, San Diego, CA), and GM-CSF (50 ng/ml; BD PharMingen) onto 24-well plates (1 ml/well) with a cell concentration of 8×10^5/ml. One-half of the medium was replaced every other day with minimal manipulation (such as by pipetting or transferring) (15). The monocytes were cultured in triplicate in complete RPMI alone or incubated with purified protein derivatives (PPD) of tuberculin (1 μg/ml; Statens Serum Institut, Copenhagen, Denmark), CMV Ag (10 μl of a 1/8 diluted solution from a commercial preparation, BioWhittaker, Walkersville, MD), or CMV control Ag (cAg) since the first day of culture (day 1). The CMV Ag preparation consisted of a commercially prepared lysate of CMV-infected fibroblasts, whereas the matched cAg consisted of a lysate of uninfected fibroblasts. In some experiments, neutralizing mAb to CD40 ligand (CD40L; 2 μg/ml, TRAP1, mouse IgG1; BD PharMingen) or purified IgG1 isotype control (BD PharMingen) was also added to cell cultures on day 1 to assess the impact of CD40-CD40L interaction on DC maturation.

Phenotypic analysis by flow cytometry

Nonadherent cells were harvested (without EDTA or trypsin) every other day (days 1, 3, 5, 7, and 9) and stained with mAb to CD11c (PE-conjugated B-ly6, mouse IgG1; BD PharMingen), CD40 (CyChrome-conjugated 5C3, IgG1), CD80 (B7.1, FITC-conjugated L307.4, IgG1), CD86 (B7.2, FITC-conjugated IT2.2, IgG2b), MHC class II (FITC-conjugated TU39, IgG2a), CD154 (CD40L, CyChrome-conjugated TRAP1, IgG1), CD3 (FITC-conjugated UCHT1, IgG1), and isotype-matched control Abs (mouse IgG1, IgG2a, and IgG2b, conjugated with compatible fluorochromes; BD PharMingen). Cells were analyzed using flow cytometry with a FACScan cytometer and CellQuest software (BD Biosciences, San Jose, CA). Baseline monocytes were also stained with mAb to CD83 (FITC-conjugated HB15a,

*Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan; and Departments of †Parasitology and ‡Medical Technology, National Taiwan University, Taipei, Taiwan

Received for publication March 27, 2001. Accepted for publication October 4, 2001.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This study was supported by Grant DOH-89-DC-1007 from the Department of Health, Taiwan, Republic of China.

[2] S.-M.H., S.-C.P., and C.-C.H. contributed equally to this work and thus are listed as co-first authors.

[3] Address correspondence and reprint requests to Dr. Szu-Min Hsieh, Laboratory of Immune Response, Section of Infectious Diseases, Department of Internal Medicine, National Taiwan University Hospital, No. 7 Chung-Shan South Road, Taipei 100, Taiwan, R.O.C. E-mail address: hsmaids@hotmail.com

[4] Abbreviations used in this paper: DC, dendritic cell; PPD, purified protein derivatives; CD40L, CD40 ligand; cAg, CMV control Ag; TST, tuberculin skin test; BrdU, bromodeoxyuridine; LDH, lactate dehydrogenase.

0022-1767/01/$02.00

IgG2b). T cells remaining in lymphocyte-depleted cell populations in day 1 culture (i.e., baseline contaminated T cells) were stained with mAb to CD45RO (PE-conjugated UCHL1, IgG2a) to differentiate their naive or memory phenotype. The cell culture was discarded if baseline contaminated T cells made up ≥1% of the harvested cell population.

Determination of levels of IL-12 production

Supernatants of the monocyte culture were harvested on day 6 and frozen at −70°C until used. Levels of IL-12 were determined using a commercial ELISA kit (Quantikine HS; R&D Systems, Minneapolis, MN) according to the instructions of the manufacturer.

Tuberculin skin test (TST)

From 48 to 72 h after intradermal injection of 1 tuberculin U PPD at the forearm, the reaction of TST was assessed by the diameter of local induration. To avoid confusion in the interpretation of an induration with a borderline diameter, we defined a positive reaction of TST as an induration diameter of >10 mm and a negative reaction as an induration diameter of <1 mm. Subjects with an induration diameter between 1 and 10 mm were excluded because of an uncertain condition of exposure.

Positive isolation of $CD4^+$ and $CD8^+$ T cells

$CD4^+$ and $CD8^+$ T cells were purified by an immunomagnetic method using a commercial kit (CD4 Positive Isolation Kit and CD8 Positive Isolation Kit; Dynabeads plus DETACHaBEAD; Dynal) according to the instructions of the manufacturer. Briefly, $CD4^+$ and $CD8^+$ T cells were positively isolated by Dynabeads and were detached from Dynabeads by DETACHaBEAD (commercially available polyclonal anti-Fab Ab specific for anti-CD4 or CD8 Ab on the Dynabeads). The resulting purity was >99% and viability was >95%.

Assessment of lymphoproliferative response of $CD4^+$ T cells

The lymphoproliferative response of $CD4^+$ T cells was assessed by determining the frequencies of $CD4^+$ T cells with bromodeoxyuridine (BrdU) incorporation after incubation with the maturing DCs (BrdU Flow Kit; BD PharMingen). Purified $CD4^+$ T cells (from a subject, 10^6 per well of a 96-well round-bottom plate) were incubated for 5 days with 5×10^4 maturing DCs in a final volume of 200 μl RPMI. The maturing DCs were from studied subjects (five CMV-seropositive, five CMV-seronegative, four TST-positive, and four TST-negative subjects) and had been incubated with CMV Ag, cAg, or PPD for 4 days (day 4 DCs) or 7 days (day 7 DCs). BrdU (final concentration, 10 μM) was added 18 h before harvest. Cells with BrdU incorporation were detected by flow cytometry after being fixed with paraformaldehyde, permeabilized with saponin, and stained with anti-BrdU-FITC according to the BrdU flow kit protocol from the manufacturer. The capacity of maturing DCs to stimulate alloreactive $CD4^+$ T cells was assessed by the lymphoproliferative response of $CD4^+$ T cells, which was calculated as frequency of $CD4^+$ T cells with BrdU incorporation (%BrdU incorporation) when stimulated by CMV Ag-incubated DCs − %BrdU incorporation when stimulated by cAg-incubated DCs, or as %BrdU incorporation when stimulated by PPD-incubated DCs − %BrdU incorporation when stimulated by DCs without Ag incubation.

Assessment of the capacity to elicit Ag-specific CTL

Monocyte-derived DCs from studied HLA-A*0201 subjects were prepared as stimulators (to elicit CTL activity) through a 7-day monocyte culture protocol with IL-4 and GM-CSF and incubation with CMV Ag or PPD. The effector cells ($CD8^+$ T cells) and targets (Ag-pulsed monocytes) were obtained from one HLA-A*0201 healthy subject who was CMV seropositive and positive for TST. The effector cells were prepared through a 5-day incubation of isolated $CD8^+$ T cells with stimulators (ratio, 5:1). Target cells were prepared by pulsing monocytes overnight with CMV Ag or cAg or PPD.

In this study, a nonradioactive assay, lactate dehydrogenase (LDH) release cytotoxicity assay kit (CytoTox 96 Non-Radioactive Cytotoxicity Assay; Promega, Madison, WI), which had been demonstrated to have results nearly identical with those determined in a parallel ^{51}Cr release assay (16, 17), was used to determine cytolytic activity according to the manufacturer's instructions. In brief, effector cells were added to quadruplicate wells with target cells with variable E:T ratios in a U-bottom 96-well plate with 10^4 targets in a final volume of 100 μl/well. Control wells for spontaneous LDH release from target cells or effector cells, culture medium background, volume correction, and maximal release from target cells were prepared according to the manufacturer's instructions. After centrifugation at 250 × *g* for 4 min, the plates were incubated for 6 h at 37°C, and then 50 μl supernatant were harvested from each well. Released LDH in supernatant was measured with a 30-min coupled enzymatic assay (provided by a CytoTox 96 kit) that resulted in the conversion of a tetrazolium salt (INT) into a red formazan product. The percentage of cytolysis was calculated as {[(experimental release − background) − (spontaneous target release − background) − (spontaneous effector release −background)]/(maximal target release − spontaneous target release)} × 100%.

CMV-specific CTL activity was calculated by subtracting the percentage of cytolysis of cAg-pulsed targets from the percentage of cytolysis of CMV Ag-pulsed ones. The PPD-specific CTL activity was calculated by subtracting the percentage of cytolysis of targets without Ag pulsing from the percentage of cytolysis of PPD-pulsed ones.

Definitions

An Ag to which a host had been recently or repetitively exposed was defined as an "exposed Ag," and an Ag to which a host had never or had only remotely been exposed was defined as an "unexposed Ag." The CMV Ag was considered to be an exposed Ag in CMV-seropositive (CMV^+) subjects, and to be an unexposed Ag in CMV-seronegative (CMV^-) subjects. PPD was considered an exposed Ag in subjects with positive TST results (TST^+), and considered an unexposed Ag in subjects whose TST results were negative (TST^-) if anergy was excluded.

Statistical analysis

The significance of differences was analyzed using a nonparametric test (Mann-Whitney *U* test) with SPSS statistical software (standard version 6.1.3; SPSS, Chicago, IL). All tests were two-tailed, and a significance level of 0.05 was used.

Results

T cell-independent model of DC maturation

To test our hypothesis, we compared the kinetics of Ag-induced DC maturation and measured the immunostimulatory capacity of these DCs when they were incubated with different Ags. First of all, to avoid the impact on DC maturation of T cell signaling via CD40-CD40L (CD154) interaction (18, 19), we created an in vitro model of T cell-independent DC maturation in which lymphocytes were highly depleted from PBMC. As a result, the baseline (day 1) contaminated T cells ranged from 0.3 to 0.9% of the harvested cell population, and T cells with memory phenotype (positive for CD45RO) ranged from 52% to 79% of baseline contaminated T cells. CD83-positive cells ranged from 0.2 to 0.8% among baseline harvested monocytes. The capacity of these baseline monocytes to elicit CMV- or PPD-specific CTL activities was undetectable. On day 5 of culture, high purity $CD11c^+$ and $CD14^-$, suspended monocyte-derived cells with typical morphology of DCs (aggregates or single cells with motile veils/dendrites), could be easily harvested for analysis (Fig. 1*a*). The T cells that remained in the cell culture system did not exceed 1% of the harvested cell population (Fig. 1*b*). These contaminated T cells rarely expressed CD40L (Fig. 1*c*). Furthermore, neutralizing Abs against CD40L, which could block the CD40-CD40L interaction, did not have a significant impact on DC maturation when compared with isotype control Abs (Fig. 1*d*). Thus, we assessed the levels of Ag-induced maturation/activation of DCs in this model free of signaling from T cells, by determining the changes in surface markers involved in their function as APCs, such as costimulatory molecules (CD80 (B7.1) and CD86 (B7.2)), a receptor for T cell signals (CD40), and Ag-presenting molecules (MHC class II molecules, such as HLA-DR), on harvested $CD11c^+$ monocytes (15, 20). When incubated with CMV Ag or PPD, $CD11c^+$ cells might mature to have the DC phenotype as demonstrated by marked up-regulation of CD80, CD86, CD40, and HLA-DR expression (Fig. 1, *e* and *f*).

The kinetics of phenotypic maturation of DCs

On day 5 of culture, levels of expression of maturation markers (CD40, CD80, CD86, and HLA-DR) on CMV Ag-incubated DCs in CMV^+ subjects were much higher than those in CMV^- subjects (Fig. 2). Similarly, levels of expression of maturation markers on

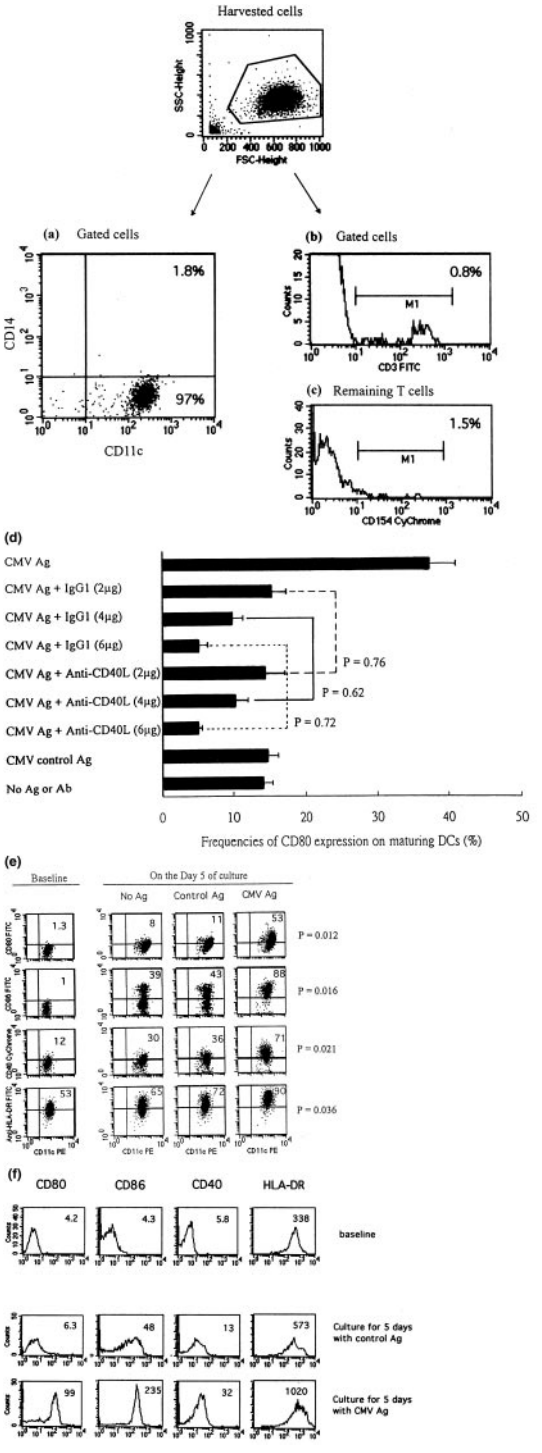

FIGURE 1. Phenotypic analysis of monocyte-derived cells incubated with IL-4, GM-CSF, and Ags after depletion of lymphocytes from PBMC and harvested on the day 5 of culture. *a*, Dead cells were excluded by forward (FSC) and side (SSC) scattering, and live cells were gated for analysis. Most (>95%) gated cells were positive for CD11c but negative for CD14. *b*, Few (<1%) contaminated $CD3^+$ T cells remained in the cell culture. *c*, CD40L (CD154) was rarely expressed (<2%) in the remaining $CD3^+$ cells. Frequencies of cells stained with matched isotype control Abs of CD11c, CD14, CD3, or CD154 were <1% (figure not shown). *d*, Neutralizing Abs to CD40L did not have a significant impact on down-regulation of CD80 expression on maturing DCs when compared with isotype control Abs (IgG1). *e*, Frequencies of expression of the maturation markers of DCs, CD80 (B7.1), CD86 (B7.2), CD40, and MHC class II (HLA-DR), were significantly up-regulated on $CD11c^+$ cells incubated with CMV Ag when compared with those incubated with cAg. Values in the right corner in each graph represent means of the frequencies of expression from five CMV-seropositive subjects. *f*, Medium fluorescein intensity of maturation marker expression is from one representative experiment of five experiments. Frequencies of cells stained with matched isotype control Abs of CD80, CD86, CD40, or HLA-DR were <1%.

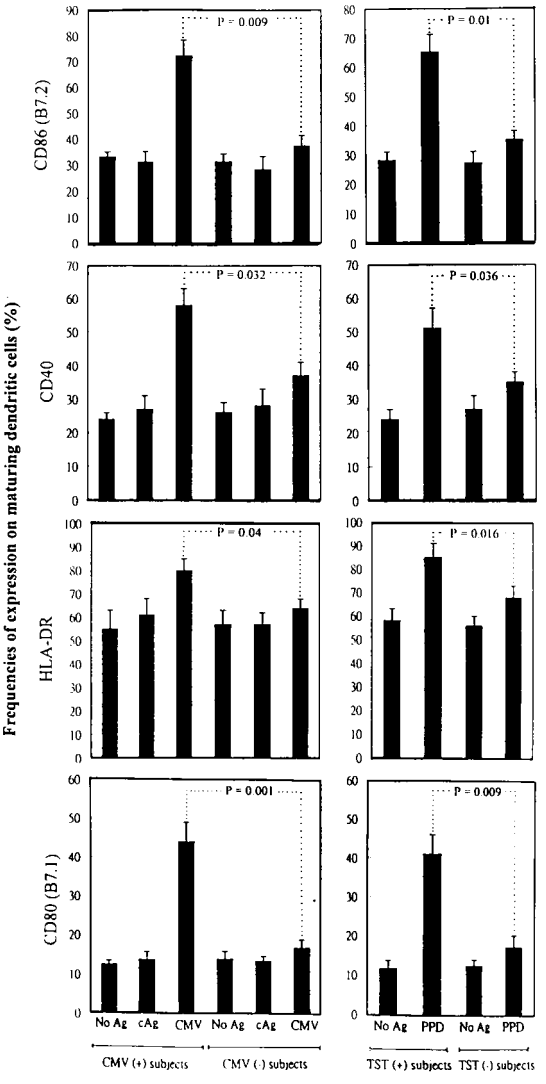

FIGURE 2. Levels of Ag-induced maturation of DCs when monocytes were incubated with exposed or unexposed Ags. CMV Ag was considered an exposed Ag in CMV-seropositive (CMV^-) subjects, and an unexposed Ag in CMV-seronegative (CMV^-) subjects. PPD was considered an exposed Ag in subjects whose TST results were positive (TST^+), and an unexposed Ag in TST^- subjects. On day 5 of culture, levels of expression of maturation markers (CD80, CD86, CD40, and HLA-DR) on CMV Ag-incubated DCs in six CMV^+ subjects were much higher than those in five CMV^- subjects (the height of the bar indicates the mean + SE). Similarly, levels of expression of maturation markers on PPD-incubated DCs in four TST^+ subjects were significantly higher than those in five TST^- subjects. Frequencies of cells stained with matched isotype control Abs of CD80, CD86, CD40, or HLA-DR were < 1%.

6PPD-incubated DCs in TST^+ subjects were significantly higher than those in TST^- subjects (Fig. 2). Furthermore, in subjects who were CMV^+ but TST^-, levels of maturation marker expression were much higher in CMV Ag-incubated DCs than in PPD-incubated DCs (Fig. 3*a*). However, in subjects who were CMV^+ and TST^+, both PPD and CMV Ag-incubated DCs up-regulated the

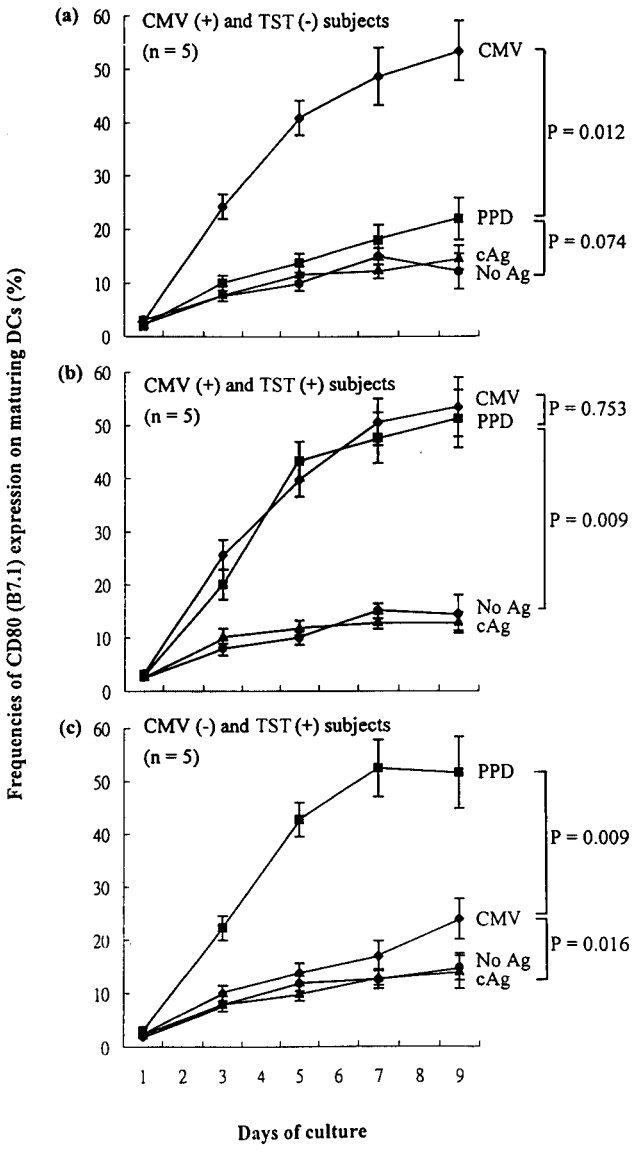

FIGURE 3. Kinetics of the phenotypic activation/maturation of maturing CD11c$^+$ DCs incubated with exposed or unexposed Ags. Nonadherent monocytes were harvested every other day (days 1, 3, 5, 7, and 9) and were analyzed by flow cytometry after staining with PE-labeled CD11c, FITC-CD80, FITC-CD86, CyChrome-CD40, and FITC-HLA-DR. Expression of all of these maturation markers showed consistent changes, and only data of CD80 expression are presented here. Both exposed and unexposed Ags up-regulated CD80 expression on maturing DCs in this culture system; however, frequencies of CD80 expression (mean ± SE of five subjects in each group) on maturing DCs in response to exposed Ags were significantly higher than those in response to unexposed Ags. Frequencies of CD80 expression for incubation with cAg and those with RPMI alone (no Ag) were similar (p = 0.35, 0.92, and 0.59, respectively). The p value represents the results of statistical comparison of frequencies of CD80 expression on maturing DCs on day 9 of culture.

expression of these maturation markers with similar patterns (Fig. 3*b*). In addition, in subjects who were CMV$^-$ but TST$^+$, the phenotypic maturation of PPD-incubated DCs was accelerated when compared with the much slower maturation in CMV Ag-incubated subjects (Fig. 3*c*). These studies were repeated at least twice using samples from the same subjects at an interval of 2–4 mo, and we found that the data were reproducible (detailed data not shown) except for the following.

One subject, who was CMV$^-$TST$^+$ in our first evaluation, was CMV$^+$TST$^+$ ~2 mo later when we repeated the study. We found that after seroconversion of CMV Ab, the expression of maturation markers on CMV Ag-incubated maturing DCs from this subject was as rapidly up-regulated as that on her PPD-incubated maturing DCs; i.e., the kinetics of Ag-induced DC maturation changed from the pattern in Fig. 3*c* to that in Fig. 3*b*. Another healthy subject who was PPD$^-$ and CMV$^+$ in the initial evaluation was PPD$^+$ ~3 mo later. The pattern of kinetics of PPD-incubated DC maturation also changed from the pattern in Fig. 3*a* to that in Fig. 3*b*.

Functional maturation of Ag-incubated DCs

To ensure that the higher levels of phenotypic activation of DCs when encountering exposed Ag in this culture system were accompanied by higher levels of functional maturation, we assessed the capacity of IL-12 production and the immunostimulatory capacity of maturing DCs. Levels of IL-12 in cell culture supernatant were determined on day 6 of culture for maturing DCs. The data showed that levels of IL-12 production from DCs incubated with CMV Ag in CMV$^+$ subjects were significantly higher than those in CMV$^-$ subjects (Fig. 4*a*). Levels of IL-12 production from DCs incubated with PPD in TST$^+$ subjects were also significantly higher than those in TST$^-$ subjects (Fig. 4*b*).

We assessed the immunostimulatory capacity of maturing DCs (stimulators) from HLA-A*0201 subjects to elicit Ag-specific CTL activity. The elicited CMV-specific CTL activity was much higher if the stimulators were cultured from monocytes from CMV$^+$ subjects than if the stimulators were from CMV$^-$ subjects (Fig. 5*a*). Similarly, PPD-specific CTL activity was much higher if the PPD-incubated stimulators were from TST$^+$ subjects than if the stimulators were from TST$^-$ subjects (Fig. 5*b*). However, a longer duration of incubation with CMV Ag may cause the stimulatory capacity of maturing DCs cultured from monocytes from CMV$^-$ subjects to approximate that from CMV$^+$ subjects (Fig. 5*c*).

We also assessed the immunostimulatory capacity of maturing DCs to elicit lymphoproliferative responses of alloreactive CD4$^+$ T cells. The lymphoproliferative responses of CD4$^+$ T cells stimulated by CMV Ag-incubated DCs (day 4 and day 7 DCs) from CMV$^+$ subjects were significantly higher than those stimulated by DCs from CMV$^-$ subjects (%BrdU incorporation: median, 3.7% vs 1.5%, p = 0.04 (day 4), and 8.1% vs 2.2%, p = 0.01 (day 7), respectively). Similarly, the responses of CD4$^+$ T cells stimulated by PPD-incubated DCs from TST$^+$ subjects were also significantly higher than those stimulated by DCs from TST$^-$ subjects (%BrdU incorporation: median, 3.3% vs 1.6%, p = 0.04 (day 4), and 7.5% vs 2.3%, p = 0.01 (day 7), respectively).

Discussion

Immunological memory, characterized by an accelerated response on the second exposure to an Ag, is an essential and exclusive character of adaptive immunity. Innate immunity occurs to the same extent no matter how many times an Ag is encountered (21). However, through the T cell-independent model, we found that human monocyte-derived DCs, a critical component of innate immunity, might have an immunological memory-like behavior. This behavior was shown by the enhanced and accelerated maturation/activation of DCs if the DC precursors encountered Ags to which they have recently or repetitively been exposed, as compared with Ags to which they have never or only remotely been exposed. This behavior of DCs may facilitate Ag processing and presentation and strengthen their costimulatory capacity to activate specific T-cells, and thus may enhance adaptive immunity of the host to fight against frequently invading pathogens from the environment in

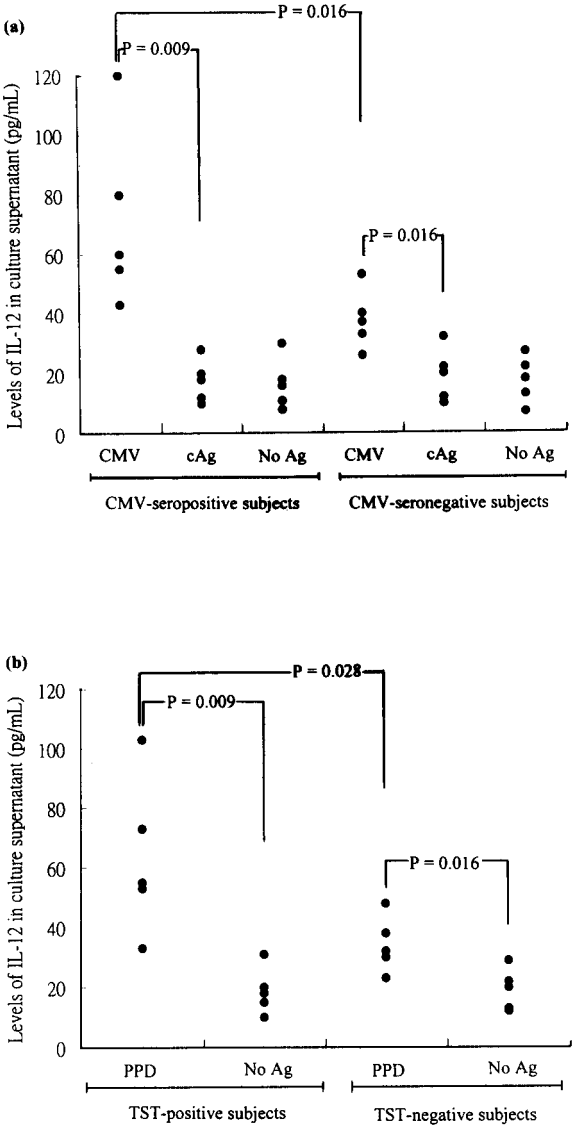

FIGURE 4. IL-12 production from maturing DCs incubated with exposed or unexposed Ags. The supernatants of the monocyte culture were harvested on day 6 of culture and frozen at −70°C until used. Levels of IL-12 were determined using a commercial ELISA kit (Quantikine™ HS, R&D Systems) *a*, Levels of IL-12 in the supernatants of culture of maturing DCs from CMV-seropositive or -negative subjects incubated with CMV Ag, cAg, or medium alone (no Ag). *b*, Levels of IL-12 in the supernatants of culture of maturing DCs from subjects positive or negative for TST incubated with PPD or without Ag.

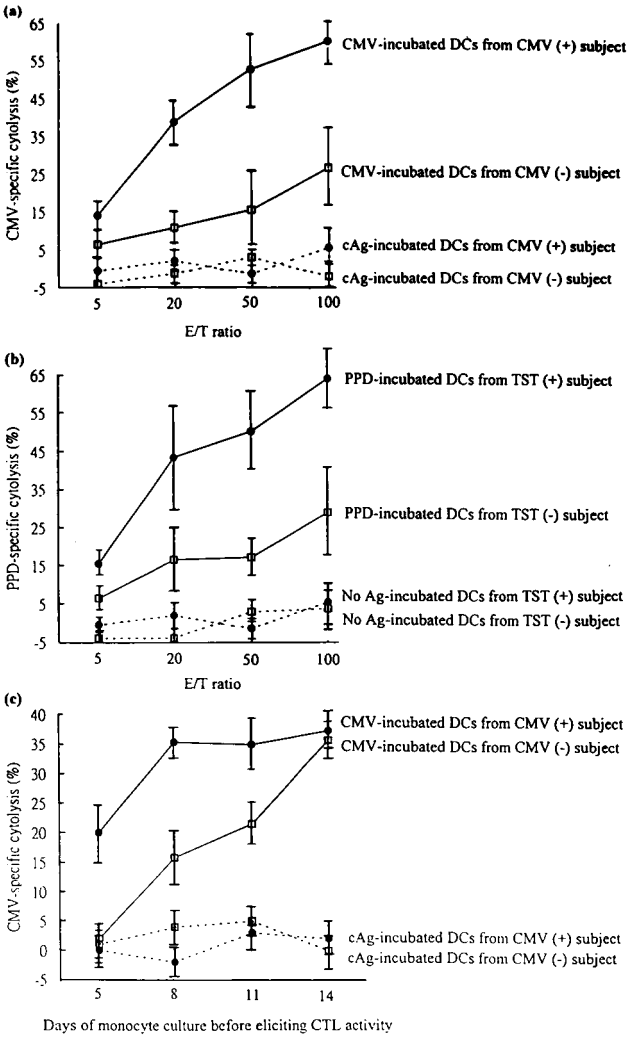

FIGURE 5. Capacity of Ag-incubated maturing DCs to elicit allogeneic $CD8^+$ CTL activity against CMV Ag or PPD. Monocyte-derived DCs from HLA-A*0201 subjects were prepared as stimulators (to elicit CTL activity) through a 7-day monocyte culture with IL-4 and GM-CSF and with CMV Ag or PPD. Effector cells ($CD8^+$ T cells) and targets (CMV Ag- or PPD-pulsed monocytes) were prepared from one HLA-A*0201 healthy subject who was CMV seropositive and positive for TST. Effector cells were prepared through a 5-day incubation of isolated $CD8^+$ T cells with stimulators (ratio, 5:1). Target cells were prepared by pulsing of monocytes overnight with Ags. *a*, Comparison of the capacity of the maturing DCs to elicit CMV-specific CTL activity from CMV-seropositive (CMV^+) and CMV-seronegative (CMV^-) subjects. *b*, Comparison of the capacity of maturing DCs to elicit PPD-specific CTL activity from subjects positive and negative for the TST. *c*, A longer duration of incubation of DCs with CMV Ag from CMV^- subjects was needed to achieve a similar capacity to elicit CMV-specific CTL activity with those from CMV^+ subjects (E:T 20). Values represent the mean of four experiments (mean ± SE).

which the host lives. This phenomenon challenges the traditional distinction between innate and adaptive immunity and may have implications for DC-based vaccine development in the future (22).

However, it is difficult to explain this phenomenon using our current knowledge of Ag recognition of innate immunity. CD14 and Toll-like receptors are the most important members of pattern recognition receptors and are essential for initiating innate responses to LPS or other microbial components including viral proteins (21, 23). However, unlike the epitope-specific receptors in T cells, these pattern recognition receptors can help APCs differentiate only if what they encounter is a foreign or self-Ag. Thus, it is unlikely that APCs can differentiate whether they have been exposed to an Ag before or not through these receptors. Thus, this phenomenon may imply that a novel Ag recognition system in innate immunity in humans may be found.

Explaining the phenomenon is further impeded because DCs appear to be programmed to die once they mature (24); thus, "memorization" of the exposure to the encountered Ag would appear to be difficult for innate immunity. However, because there is evidence that mature DCs may receive survival signals from T cells to avoid the fate of apoptosis (25), perhaps a minor fraction of these mature/activated DCs remain alive with some kind of "memory phenotype," or become precursors in vivo as sentinels especially for repetitively encountered Ags.

This phenomenon may be explained by the existence of a subpopulation of transitional stage DCs or some mature DCs in our

starting cell population. However, we think that this possibility is small. First, there were only minimal mature DCs in our starting cell population (<1% of baseline monocytes were CD83 positive). Second, the existence of a few mature or transitional stage DCs may partially explain the results of functional assays but may be difficult to explain the accelerated up-regulation of expression of maturation markers of DCs (such as CD80, CD86, CD40, and HLA-DR) on cultured monocytes when they encounter exposed Ags.

Because DC maturation can be primed by T cell signaling through CD40-CD40L interaction (18, 19), the contaminated T cells in monocyte culture might have contributed to the maturation of DCs. However, in this study, <1% of cultured cell population were T cells. These contaminated T cells could not fully explain the rapid maturation of DCs because these T cells failed to express CD40L, and anti-CD40L-neutralizing Abs failed to influence DC maturation (when compared with isotype control). The failure to express CD40L on contaminated T cells might be due to the effect of a high dose of IL-4 or the lack of IL-2 in the culture system (26). Thus, we think that contaminated T cells should not have a significant impact on the accelerated functional and phenotypic maturation of DCs when monocytes encounter exposed Ags.

To establish the culture environment, we negatively isolated monocytes from PBMC rather than positively purified monocytes because the binding of anti-CD14 mAbs, which would be used in positive sorting, would activate the isolated monocytes (27). Thus, negatively isolated monocytes could remain untouched and free of surface-bound Ab and/or beads and were more suitable for observation of the kinetics of Ag-induced activation/maturation ex vivo.

According to our data, we propose a novel role of innate immunity in humans; i.e., when a host is invaded by a pathogen, innate immunity may not only respond immediately (e.g., phagocytosis and/or cytokine release) after recognizing the foreign materials by pattern recognition receptors but also accelerate the maturation and enhance the immunostimulatory capacity of DCs to activate Ag-specific T cells earlier and more effectively if the pathogen has invaded the host before. Thus, maximal host protection from invasion of frequently encountered pathogens in the environment can be achieved.

Acknowledgments

We thank David D. Ho and Xia Jin (Aaron Diamond AIDS Research Center, Rockefeller University) for their valuable comments on the manuscript.

References

1. Ahmed, R., and D. Gray. 1996. Immunological memory and protective immunity: understanding their relation. *Science 272:54.*
2. Delves, P. J., and I. M. Roitt. 2000. The immune system (first of two parts). *N. Engl. J. Med. 343:37.*
3. Bell, D., J. W. Young, and J. Banchereau. 1999. Dendritic cells. *Adv. Immunol. 72:255.*
4. Banchereau, J., and R. M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature 392:245.*
5. Sallusto, F., and A. Lanzavecchia. 1994. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor α. *J. Exp. Med. 179:1109.*
6. Romani, N., S. Gruner, D. Brang, E. Kampgen, A. Lenz, B. Trockenbacher, G. Konwalinka, P. O. Fritsch, R. M. Steinman, and G. Schuler. 1994. Proliferating dendritic cell progenitors in human blood. *J. Exp. Med. 180:83.*
7. Palucka, K., and J. Banchereau. 1999. Dendritic cells: a link between innate and adaptive immunity. *J. Clin. Immunol. 19:12.*
8. Celluzzi, C. M., J. I. Mayordomo, W. J. Storkus, M. T. Lotze, and L. D. Falo. 1996. Peptide-pulsed dendritic cells induce antigen-specific CTL-mediated protective tumor immunity. *J. Exp. Med. 183:283.*
9. Fearon, D. T., and R. M. Locksley. 1996. The instructive role of innate immunity in the acquired immune response. *Science 272:50.*
10. Moser, M., and K. M. Murphy. 2000. Dendritic cell regulation of Th1-Th2 development. *Nat. Immunol. 1:199.*
11. Langenkamp, A., M. Messi, A. Lanzavecchia, and F. Sallusto. 2000. Kinetics of dendritic cell activation: impact on priming of Th1, Th2 and nonpolarized T cells. *Nat. Immunol. 1:311.*
12. Cella, M., F. Facchetti, A. Lanzavecchia, and M. Colonna. 2000. Plasmacytoid dendritic cells activated by influenza virus and CD40L drive a potent TH1 polarization. *Nat. Immunol. 1:305.*
13. Bhardwaj, N., A. Bender, N. Gonzalez, L. K. Bui, M. C. Garrett, and R. M. Steinman. 1994. Influenza virus-infected dendritic cells stimulate strong proliferative and cytolytic responses from human $CD8^+$ T cells. *J. Clin. Invest. 94:797.*
14. Hsieh, S. M., C. C. Hung, S. C. Pan, T. T. Wang, S. C. Tsai, M. Y. Chen, and S. C. Chang. 2000. Restoration of cellular immunity against tuberculosis in HIV-1/tuberculosis co-infected patients with effective antiretroviral therapy: assessment by determination of CD69 expression on T-lymphocytes after tuberculin stimulation. *J. AIDS 25:212.*
15. Gallucci, S., M. Lolkema, and P. Matzinger. 1999. Natural adjuvants: endogenous activators of dendritic cells. *Nat. Med. 5:1249.*
16. Korzeniewski, C., and D. M. Callewaert. 1983. An enzyme-release assay for natural cytotoxicity. *J. Immunol. Methods 64:313.*
17. Decker, T., and M. L. Lohmann-Matthes. 1988. A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor activity. *J. Immunol. Methods 115:61.*
18. Shreedhar, V., A. M. Moodycliffe, S. E. Ullrich, C. Bucana, M. L. Kripke, and L. Flores-Romo. 1999. Dendritic cells require T cells for functional maturation in vivo. *Immunity 11:625.*
19. Liu, Z., S. Tugulea, R. Cortesini, S. Lederman, and N. Suciu-Foca. 1999. Inhibition of CD40 signaling pathway in antigen presenting cells by T suppressor cells. *Hum. Immunol. 60:568.*
20. Palucka, K., N. Taquet, F. Sanchez-Chapuis, and J. C. Gluckman. 1998. Dendritic cells are the terminal stage of monocyte differentiation. *J. Immunol. 160:4587.*
21. Medzhitov, R., and C. Janeway. 2000. Innate immunity. *N. Engl. J. Med. 343:338.*
22. Tarte, K., and B. Klein. 1999. Dendritic cell-based vaccine: a promising approach for cancer immunotherapy. *Leukemia 13:653.*
23. Kurt-Jones, E. A., L. Popova, L. Kwinn, L. M. Haynes, L. P. Jones, R. A. Tripp, E. E. Walsh, M. W. Freeman, D. T. Golenbock, L. J. Anderson, and R. W. Finberg. 2000. Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus. *Nat. Immunol. 1:398.*
24. De Smedt, T., B. Pajak, G. G. Klaus, R. J. Noelle, J. Urbain, O. Leo, and M. Moser. 1998. Antigen-specific T-lymphocytes regulate lipopolysaccharide-induced apoptosis of dendritic cells in vivo. *J. Immunol. 161:4476.*
25. Wong, B. R., R. Josien, S. Y. Lee, B. Sauter, H. L. Li, R. M. Steinman, and Y. Choi. 1997. TRANCE (tumor necrosis factor [TNF]-related activation-induced cytokine), a new TNF family member predominantly expressed in T cells, is a dendritic cell-specific survival factor. *J. Exp. Med. 186:2075.*
26. Skov, S., M. Bonyhadi, N. Odum, and J. A. Ledbetter. 2000. IL-2 and IL-15 regulate CD154 expression on activated CD4 T cells. *J. Immunol. 164:3500.*
27. Lund-Johansen, F., J. Olweus, F. W. Symington, A. Arli, J. S. Thompson, R. Vilella, K. Skubitz, and V. Horejsi. 1993. Activation of human monocytes and granulocytes by monoclonal antibodies to glycosylphosphatidylinositol-anchored antigens. *Eur. J. Immunol. 23:2782.*