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# Acemannan purified from *Aloe vera* induces phenotypic and functional maturation of immature dendritic cells

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**Abstract**

Acemannan, a major carbohydrate fraction of *Aloe vera* gel, has been known to have antiviral and antitumoral activities in vivo through activation of immune responses. The present study was set out to define the immunomodulatory activity of acemannan on dendritic cells (DCs), which are the most important accessory cells for the initiation of primary immune responses. Immature DCs were generated from mouse bone marrow (BM) cells by culturing in a medium supplemented with GM-CSF and IL-4, and then stimulated with acemannan, sulfated acemannan, and LPS, respectively. The resultant DCs were examined for phenotypic and functional properties. Phenotypic analysis for the expression of class II MHC molecules and major co-stimulatory molecules such as B7-1, B7-2, CD40 and CD54 confirmed that acemannan could induce maturation of immature DCs. Functional maturation of immature DCs was supported by increased allogeneic mixed lymphocyte reaction (MLR) and IL-12 production. The differentiation-inducing activity of acemannan was almost completely abolished by chemical sulfation. Based on these results, we propose that the adjuvant activity of acemannan is at least in part due to its capacity to promote differentiation of immature DCs. 

*Keywords:* Aloe vera; Acemannan; Dendritic cell; Differentiation

## 1. Introduction

Acemannan is a mixture of polymers of various length chains of $\beta$-(1,4)-linked acetylated mannan isolated from the gel of Aloe species plants [1]. Acemannan has been known to have diverse immunomodulatory activities in vivo as well as in vitro (reviewed in Ref. [2]). When administered intraperitoneally to tumor-implanted mice, acemannan was shown to cure completely or reduce the tumor bur-

*Abbreviations:* ACM, acemannan; BM, bone marrow; DC, dendritic cell; GM-CSF, granulocyte macrophage-colony stimulating factor; IL, interleukin; LPS, lipopolysaccharide; MLR, mixed lymphocyte reaction; MFI, mean fluorescence intensity; S-ACM, sulfated acemannan; TNF-$\alpha$, tumor necrosis factor-$\alpha$

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den significantly [3]. Acemannan has also been shown to be effective in the treatment of spontaneously developed canine and feline fibrosarcomas [4,5]. The antitumor activity of acemannan is thought to be mediated via activation of the host defense mechanisms, and not by the direct cytotoxicity to tumor cells. Immunoaugmenting activities of acemannan have also been demonstrated in numerous other systems. Acemannan has been shown to increase lymphocyte responses to alloantigens in vitro [6,7], exhibit adjuvant activity in vaccination against virus [8] or heart-worm antigen [9], and increase survival rate in virus-infected animals [10,11].

Some of the immunoaugmenting activities of acemannan appeared to be mediated through activation of macrophages. Acemannan was shown to activate macrophage to produce inflammatory cytokines such as IL-1, IL-6 and TNF-$\alpha$ [6,7,12]. Acemannan, in the presence of IFN-$\gamma$, was also shown to markedly increase NO production by macrophages [12–14], and upregulate phagocytic and candidicidal activities [15]. Acemannan may exert these immunomodulatory activities via binding to mannose receptors on the macrophages [13,16].

Numerous reports in recent years have documented that dendritic cells (DCs) are the most important accessory cells for the activation of naïve T cells and generation of primary T cell responses (reviewed in Ref. [17]). Resident DCs, however, are least efficient in performing these functions, and must be activated and differentiated further by microenvironmental signals. Maturated and differentiated DCs are distinguished from immature DCs in that they express high levels of class II MHC molecules and co-stimulatory molecules such as B7-1, B7-2, CD40 and CD54, and exhibit strong allo-stimulatory activities [17]. Because DCs also have mannose receptors [18–20], acemannan may bind to DCs and exhibit immunomodulatory activity through DC mannose receptors.

In this study, we examined the effects of acemannan on immature DCs. Immature DCs were generated from mouse bone marrow cells, and then cultured with acemannan, sulfated acemannan and LPS, respectively. Functional and phenotypic analysis of the resultant DCs showed that acemannan, but not the sulfated acemannan, could effectively induce maturation of immature DCs. Our results may provide explanation for some of the immunomodulatory activities of acemannan.

## 2. Materials and methods

### 2.1. Isolation and characterization of acemannan

Acemannan was isolated from the gel of *Aloe vera* as described previously [21]. Briefly, the freeze-dried gel, provided from Aloecorp (Harlingen, TX, USA), was dissolved in distilled water, and then fractionated according to the molecular size using a ultrafiltration cell from Amicon (Beverly, MA, USA) inserted with the respective molecular weight cut-off membrane. The molecular weight of the finally purified acemannan fraction was determined by HPLC on GPC column of BIOSEP SEC H400 ($0.75 \times 60$ cm) from Phenomenex (Torells, CA, USA) equilibrated with 100 mM NaCl at a flow rate of 1.0 ml/min. Our preparation of acemannan at a concentration of 100 $\mu$g/ml contained less than 0.125 ng/ml of endotoxin when determined by a limulus amoebocyte assay (Associates of Cape Cod, Woods Hole, MA, USA).

Monosaccharide composition was analyzed by a gas chromatography. Briefly, 5 mg of acemannan was hydrolyzed, reduced and acetylated according to the previous report [22]. Acetylated alditols of sugars were separated on a SP 2330 glass-capillary column (15 m $\times$ 0.25 mm) in a Hewlett-Packard 5730A chromatography equipment with a flame ionization detector as described previously [23].

For $^{1}$H NMR spectroscopy, approximately 1 mg of the sample was exchanged three times with 0.5 ml portions of $^{2}H_2O$, followed by in vacuo desiccation over $P_2O_5$. The thoroughly dried sample was re-dissolved in 0.7 ml of $^{2}H_2O$ (99.96%) and the spectrum was obtained using a Brucker AMX5 spectrometer at the operating frequency of 500 MHz equipped with VAX 32 computer at the Korea Basic Science Center.

### 2.2. Chemical oversulfation of acemannan

Sulfated acemannan was prepared as described previously [24]. Acemannan (150 mg) was suspended in anhydrous pyridine (5 ml). To this suspen-

sion, 5 ml of a mixture of chlorosulfonic acid and pyridine (1:6) was added and the reaction was refluxed for 2 h. The solution was cooled, mixed with 10 ml of ice-cold water, and its pH was adjusted to 7.0 with 2 M sodium hydroxide. Three volumes of ethanol was then added and the mixture was left overnight at 4°C. The precipitates formed were recovered by centrifugation, dissolved in water, dialyzed exhaustively against water and freeze-dried.

### 2.3. Cell culture

Cells were cultured in Dulbecco's modified Eagle's medium (Gibco BRL) supplemented with 10% heat-inactivated fetal bovine serum (Hyclone), 2 mM glutamine, 100 U/ml penicillin and 100 $\mu$g/ml streptomycin (Gibco BRL), 1% (v/v) nonessential amino acids, and 50 $\mu$M 2-mercaptoethanol at 37°C, 5% $CO_2$ condition.

### 2.4. Mitogenic activity

Total spleen cells were prepared from spleen of C57BL/6 mouse by gentle disruption between two notched slide glasses. The single cell suspension was washed with PBS, and then red blood cells were lysed by treatment with ACK lysis buffer (0.15 M $NH_4Cl$, 1.0 mM $KHCO_3$, 0.1 mM EDTA) for 3 min. The spleen cells were washed, and then cultured in U-bottom well plates ($2 \times 10^5$/well) in a volume of 200 $\mu$l per well in the presence or absence of different concentrations of stimuli for 3 days. DNA synthesis was measured by $^3$H-thymidine (Du Pont) incorporation (1 $\mu$Ci/well) for the final 6 h of the culture period.

### 2.5. Generation of immature DCs

Immature DCs were generated from BM cells of C57BL/6 mice as described previously [25]. Briefly, total BM cells obtained from femurs of mice were cultured in a six-well plate ($5 \times 10^6$/well) in a culture medium (3 ml/well) supplemented with 200 U/ml rmGM-CSF (PeproTech, Rocky Hill, NJ) and 100 U/ml rmIL-4 (PeproTech). At days 2 and 4 from the initiation of the culture, nonadherent cells were discarded by replacing the culture medium with fresh medium containing the cytokines after gentle shaking. Aggregates of immature DCs were apparent at day 6, and were used for the maturation experiments.

### 2.6. Induction of maturation of DCs

At day 6 from the initiation of culture, each well of immature DC cultures was fed with 2 ml of fresh medium with the same cytokines (total volume, 5 ml). For induction of maturation, various concentrations of acemannan, sulfated acemannan or LPS were added to each well in a volume of 50 $\mu$l, and cultured for additional 2 days. The culture supernatants and the cells were then collected separately after gentle pipetting, and used for the subsequent experiments. For blocking experiments, D-(+)-mannose (Janssen Chimica, Beerse, Belgium) was added to the immature DC cultures (5 mg/ml) together with acemannan.

### 2.7. Phenotypic analysis

Cells were stained with monoclonal antibodies recognizing murine cell surface markers as described previously [26], and flow cytometric analysis was performed on a FACS Caliver (Becton-Dickinson). The monoclonal antibodies, anti-CD40 (clone 3/23), anti-ICAM-1 (clone 3E2), anti-I-$A^b$ (clone AF6-120.1), anti-B7-1 (clone 16-10A1), anti-B7-2 (clone GL1), and isotype-matched control antibodies were purchased from Pharmingen (San Diego, CA). Dead cells were gated out by their low forward angle light scatter intensity. In most analysis, 10,000 cells were scored.

### 2.8. Allogenic mixed lymphocyte reaction (MLR)

All DC populations were treated with mitomycin C (Sigma) for 20 min at 37°C, washed three times with culture medium, and then adjusted to $1 \times 10^5$–$2 \times 10^5$/ml. Responder T cells were isolated from spleen of Balb/c mouse by a nylon wool-enrichment technique, and adjusted to $2 \times 10^6$/ml. Each 100 $\mu$l cell suspension was mixed and cultured in 96-well U-bottom plates for 4 days. DNA synthesis was measured by $^3$H-thymidine (Du Pont) incorporation (1 $\mu$Ci/well) for the final 6 h of the culture period.

### 2.9. ELISA for IL-12

The IL-12 levels in the culture supernatants of stimulated DCs were measured by a commercial immunoassay kit (R&D System) that detects the bioactive p70 heterodimer.

## 3. Results

### 3.1. Isolation and chemical oversulfation of acemannan

The yield of acemannan from the lyophilized gel of *Aloe vera* was about 2%. The average molecular weight of the purified acemannan was larger than 500,000 Da (data not shown). Monosaccharide compositional analysis of the purified acemannan using gas chromatography indicated that it was composed of 97% of mannose and 3% of glucose. Acetylation was confirmed by IR spectrum. The stretching bands of carbonyl groups were clearly shown at 1236 and 1736 $cm^{-1}$. The bands were disappeared after alkaline hydrolysis. The multiple peaks in the NMR spectrum demonstrated that the molecule contained acetyl groups. The NMR spectrum of the sample was consistent with that of acemannan reported previously [1].

Chemical sulfation of acemannan was performed in pyrimidine solution with chlorosulfonic acid as described in detail in Materials and methods. The sulfate level of the structurally modified acemannan ranged from 30% to 35% (w/w).

### 3.2. Mitogenic activities of acemannan and sulfated acemannan

Our preparation of acemannan did not show mitogenic activity at a concentration of 10 $\mu$g/ml when added to the culture of mouse total spleen cells (Fig. 1). However, acemannan was obviously mitogenic at a higher concentration (100 $\mu$g/ml). The mitogenic activity of the sulfated acemannan was also examined in the culture of mouse total spleen cells. As shown in Fig. 1, sulfated acemannan did not show statistically significantly different mitogenic activity even at the concentration of 100 $\mu$g/ml. Thus, it appears that chemical sulfation abolishes mitogenic activity almost completely.

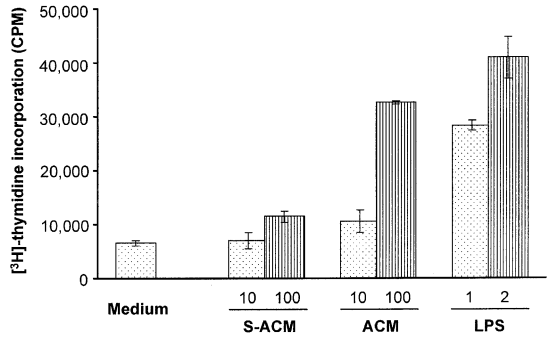



Fig. 1. Mitogenic activity of acemannan (ACM) and sulfated acemannan (S-ACM). Acemannan was isolated from *Aloe vera* gel, and then chemically sulfated in pyrimidine solution with chlorosulfonic acid as described in Materials and methods. Mitogenic activities of acemannan and sulfated acemannan were examined in two different concentrations, 10 and 100 $\mu$g/ml, in cultures of mouse total spleen cells ($2\times10^5$/well). LPS (1 and 2 $\mu$g/ml) was used as a control. DNA synthesis was measured by $^3$H-thymidine incorporation for the final 6 h of the culture period of 3 days. Each data point represents the mean $\pm$ SD of values obtained from three individual experiments.

### 3.3. Phenotypic maturation of immature DCs by acemannan

The effects of acemannan and sulfated acemannan on the induction of maturation of immature DCs were examined in vitro. For these experiments, immature DCs were generated from mouse BM cells by culturing for 6 days in the presence of GM-CSF and IL-4. Immature DCs were then stimulated with 10 and 100 $\mu$g/ml of acemannan, sulfated acemannan, or LPS, respectively, in the presence of GM-CSF and IL-4 for 2 days. After 2 days of stimulation, DCs were harvested and used for phenotypic analysis. As shown in Fig. 2, control DCs that were cultured in a medium containing GM-CSF and IL-4 (without extra stimulus) revealed characteristics of the most immature DCs by the criteria of staining with MHC class II (I-A), B7-1, B7-2 and CD40 molecules. In contrast, the DCs stimulated with acemannan revealed more matured phenotypes. This phenotypic maturation-inducing activity of acemannan was dose dependent. While the DCs stimulated with 10 $\mu$g/ml of

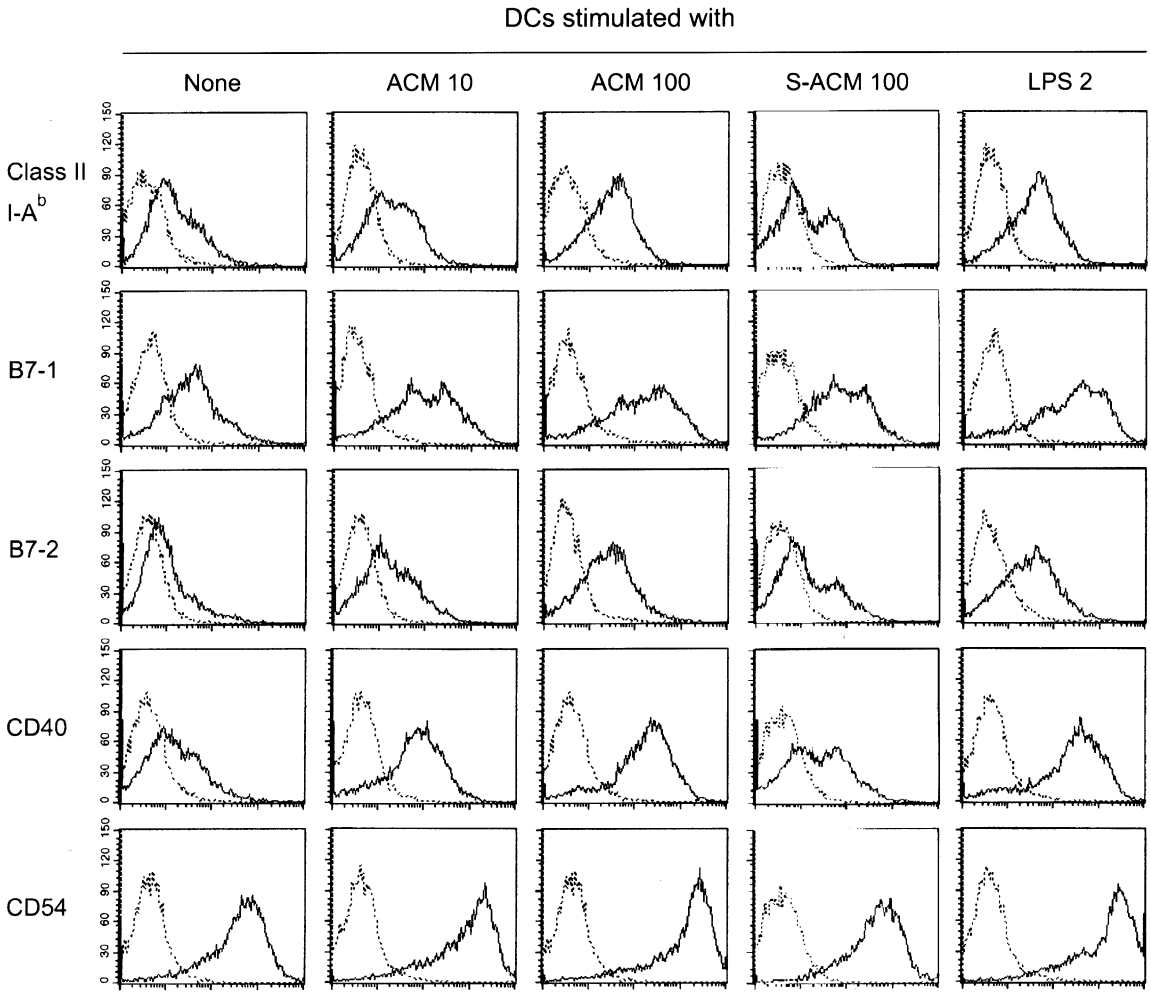



Fig. 2. Induction of phenotypic maturation of DCs by acemannan. Immature DCs were generated from mouse BM cells by culturing for 6 days in a medium supplemented with GM-CSF and IL-4. Immature DCs were then stimulated with ACM (10 and 100 $\mu$g/ml), S-ACM (100 $\mu$g/ml), and LPS (2 $\mu$g/ml), respectively, for 2 days. The resultant DCs were used for immunophenotypic analysis. Immunophenotypic profiles of DCs are shown compared to isotype controls.

acemannan exhibited intermediate level of maturation phenotypes, the DCs stimulated with 100 $\mu$g/ml of acemannan exhibited more differentiated phenotypes (Fig. 2). Mean fluorescence intensity (MFI) values were calculated from two or three independent experiments and were shown in Table 1. As shown in Table 1, the MFI values of class II MHC molecules and major costimulatory molecules were increased in order of stimulation with 10 $\mu$g/ml of acemannan, 100 $\mu$g/ml of acemannan, and 2 $\mu$g/ml of LPS. Sulfated acemannan did not show discernible effect on phenotypic changes of immature DCs at a concentration of 10 $\mu$g/ml (data not shown). At a higher concentration (100 $\mu$g/ml), sulfated acemannan induced phenotypic changes of immature DCs, but at a level lower than that induced by 10 $\mu$g/ml of acemannan (Fig. 2 and Table 1).

### *3.4. Functional maturation of immature DCs by acemannan*

The same populations of stimulated DCs that were used for phenotypic analysis in Fig. 2 were also

Table 1
Mean fluorescence intensity of the DCs

| Surface antigen | Stimulation of the immature DCs with | | | | |
|---|---|---|---|---|---|
| | None | ACM 10 | ACM 100 | S-ACM | LPS 2 |
| Ia | 17.5 ± 2.7 | 27.8 ± 3.7 | 45.6 ± 2.5 | 25.25 ± 5.1 | 55.6 ± 1.9 |
| B7-1 | 64.1 ± 7.8 | 244.5 ± 9.1 | 388.3 ± 5.5 | 131.5 ± 3.2 | 670.5 ± 6.3 |
| B7-2 | 12.0 ± 3.9 | 31.3 ± 2.7 | 41.3 ± 4.1 | 29.5 ± 1.9 | 61.2 ± 5.3 |
| CD40 | 31.7 ± 3.1 | 115.4 ± 9.4 | 343.3 ± 11.2 | 72.59 ± 3.8 | 694.8 ± 17.8 |

Note: Acemannan treatment upregulates surface expression on DCs. In this experiment, immature DCs generated from BM cells were stimulated with ACM (10 and 100 μg/ml), S-ACM (100 μg/ml), and LPS (2 μg/ml), respectively, for 2 days. Immunophenotypic profiles of the resultant DCs were analyzed as in Fig. 2. Numbers indicate the mean ± SD of mean fluorescence (MFI) values obtained from two or three individual experiments. The MFI values of cells stained with isotype-matched control antibodies were 3.8 ± 0.4.

used as stimulators in the allogeneic MLR. As shown in Fig. 3, control DCs that were cultured in a medium containing GM-CSF and IL-4 (without extra stimulus) exhibited low to moderate level of stimulatory capacity for the proliferation of allogeneic T cells. However, DCs that were stimulated with acemannan in the presence of GM-CSF and IL-4 exhibited profoundly enhanced allostimulatory capacity compared with the control DCs. This differentiation-inducing activity of acemannan on immature DCs was increased in a dose-dependent manner, and was lost almost completely by sulfation. Since all the DCs were treated with mitomycin C, and washed thoroughly to remove mitomycin C, the difference in the T cell proliferation must be the reflection of difference in DC accessory cell function, and is not due to the mitogenic activity of contaminating acemannan.

### *3.5. Effects of mannose on the differentiation-inducing activity of acemannan*

In order to examine whether acemannan uses mannose receptor to promote maturation of immature DCs, we added mannose to the culture to address this issue. As shown in Fig. 4A, addition of mannose (5 mg/ml) to the culture of mouse spleen cells inhibited the mitogenic activity of acemannan almost completely. Thus, same concentration of

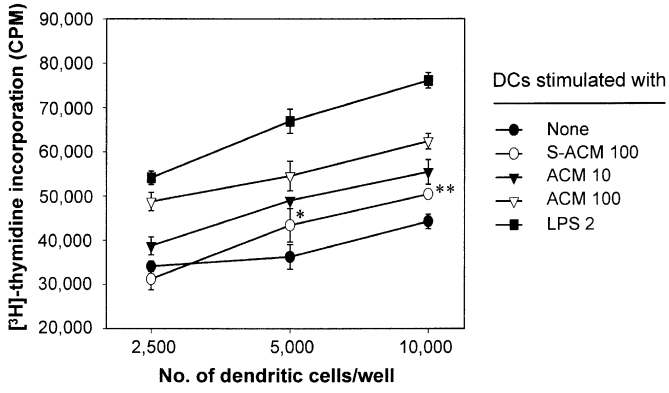
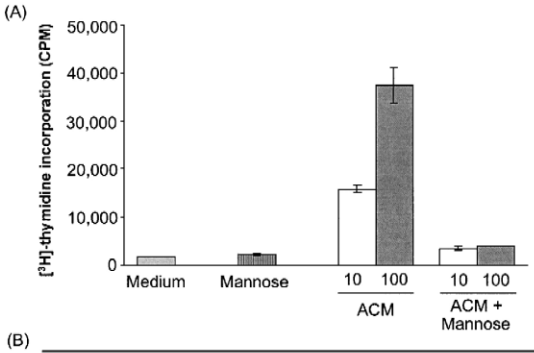



Fig. 3. Allostimulatory capacity of DCs cultured with acemannan. Immature DCs generated from mouse BM cells were stimulated with ACM (10 and 100 μg/ml), S-ACM (100 μg/ml) and LPS (2 μg/ml), respectively, for 2 days. The resultant DCs were treated with mitomycin C, washed, and then used as stimulators in allogeneic MLR. Proliferation of T cells in the allogeneic MLR was measured by $^{3}$H-thymidine incorporation for the final 6 h of the culture period of 4 days. Each data point represents the mean ± SD of values obtained from three individual experiments. $^{*}P > 0.05$ compared to the unstimulated control, $^{**}P < 0.05$ compared to the unstimulated control.

mannose was added to the immature DC culture at the same time point with acemannan. After 2 days of incubation, the resultant cells were analyzed for phenotypic and functional properties. As shown in Fig. 4B, addition of mannose did not suppress the




(B)

| Surface antigen | Stimulation of the immature DCs with | | | |
|---|---|---|---|---|
| | None | Mannose | ACM100 | ACM100 + Mannose |
| Ia | 83.75 ± 3.9 | 84.89 ± 8.1 | 131.53 ± 12.2 | 120.08 ± 23.0 |
| B7-1 | 231.9 ± 18.7 | 209.9 ± 14.1 | 537.11 ± 36.1 | 473.46 ± 32.1 |
| B7-2 | 109.41 ± 9.2 | 111.6 ± 3.2 | 204.8 ± 21.1 | 215.7 ± 14.9 |
| CD40 | 121.1 ± 9.7 | 122.4 ± 4.8 | 426.6 ± 32.2 | 380.7 ± 13.7 |
| CD54 | 697.22 ± 28.1 | 689.41 ± 17.4 | 1903.52 ± 41.1 | 831.35 ± 21.2 |

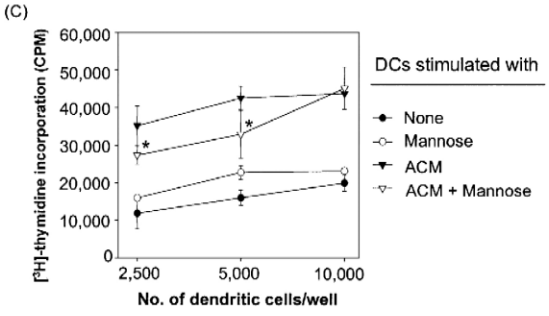



Fig. 4. Effect of mannose on the biological activity of acemannan. (A) Mitogenic activities of ACM (10 and 100 $\mu$g/ml) were examined in the absence or presence of 5 mg/ml of mannose in the culture of mouse total spleen cells ($2\times10^5$/well). (B) Immature DCs generated from mouse BM cells were stimulated with acemannan (ACM 100 $\mu$g/ml) in the absence or presence of 5 mg/ml of mannose for 2 days. The resultant DCs were used for immunophenotypic analysis. Numbers indicate the mean ± SD of mean fluorescence (MFI) values. (C) The DCs generated with acemannan in the absence or presence of 5 mg/ml of mannose were also used as stimulators in allogeneic MLR. $^{*}P > 0.05$ compared to the 'ACM 100' group.

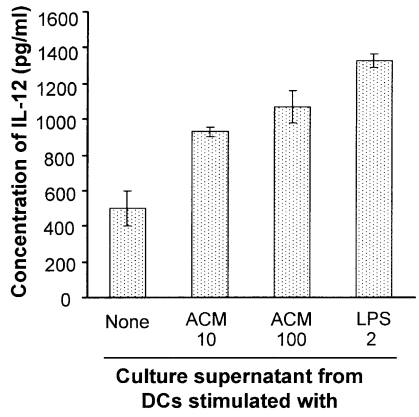



Fig. 5. Production of IL-12 by DCs cultured with acemannan. The culture supernatants of the DCs stimulated as described in Fig. 2 were collected, and assayed for the levels of IL-12. IL-12 concentration was measured by a commercial immunoassay kit that detects the bioactive p70 heterodimer according to the manufacturer's instruction.

acemannan-induced expression of class II MHC molecules and major co-stimulatory molecules such as B7-1, B7-2, and CD40. The only significantly suppressed surface molecule was CD 54 (Fig. 4B). Mannose also did not suppress the allostimulatory capacity of DCs stimulated with acemannan (Fig. 4C).

### *3.6. Induction of IL-12 production by acemannan*

Interleukin-12 produced by DCs is a critical cytokine for the induction of cell-mediated immune responses [27]. Thus, we examined whether acemannan could also induce the production of IL-12 from DCs. As shown in Fig. 5, acemannan induced IL-12 production significantly when added to the culture of immature DCs. The amount of IL-12 in the culture supernatant of acemannan (100 $\mu$g/ml)-stimulated DCs reached up to 1100 pg/ml, while the amount of IL-12 in the culture supernatant of control DCs, which was cultured with GM-CSF and IL-4, was 490 pg/ml.

## 4. Discussion

Our results demonstrate that acemannan induces maturation of DCs in vitro. When added to cultures

of immature DCs, acemannan induced phenotypic maturation evidenced by up-regulation of class II MHC molecules and co-stimulatory molecules such as B7-1, B7-2, CD40 and CD54. Acemannan also induced functional maturation evidenced by increased allogeneic T cell stimulatory activity and production of IL-12.

The immature DCs used in the present study were generated from mouse BM cells by culturing in a medium supplemented with GM-CSF and IL-4 based on the methods described previously [25]. The concentration of GM-CSF and IL-4, however, was lowered optimally through several pilot experiments to maximize the recovery of immature DCs. Immature DCs developed at day 6 from the initiation of culture were always used for the stimulation experiments with acemannan. The DCs developed in our culture condition were mostly immature ones even at day 8 as revealed by low expression of class II MHC molecules and co-stimulatory molecules, and poor allostimulatory capacity ('control DCs' in Figs. 2 and 3).

DCs play a key role in the initiation of primary immune responses (reviewed in Ref. [17]). DCs can acquire and process antigens in the periphery, and migrate to secondary lymphoid tissues where they prime primary T cell responses. The capability of DCs to activate even naïve T cells in a primary response has been explained by their ability to express high levels of class II MHC and costimulatory molecules and synthesize cytokines such as IL-12. The DCs residing in tissues and organs of the body, however, do not express high levels of costimulatory molecules and are poor T cell stimulators [28], and thus are sometimes referred to as immature DCs [29,30]. These immature DCs must be matured or activated further to fully perform accessory cell functions. Maturation signals appear to be very diverse, and include inflammatory cytokines such as TNF-$\alpha$ and IL-1, CD40-CD40 ligand interactions, simple chemicals such as 2,4-dinitrochlorobenzene (DNCB), bacteria, and bacterial products such as LPS (reviewed in Refs. [31,32]). In the present study, we identified that acemannan, which has long been known to activate host immune responses, can also induce maturation of immature DCs. This DC maturation-inducing activity of acemannan was dose dependent. The DCs cultured with higher concentration of acemannan (100 $\mu$g/ml) exhibited more potent allogeneic T cell stimulatory activity compared with those cultured with lower concentration (10 $\mu$g/ml) of acemannan. This functional difference was exactly correlated with the differences in the expression levels of class II MHC and costimulatory molecules between two groups. We also observed that in the presence of acemannan (100 $\mu$g/ml), growth of the aggregates of immature DCs ceased and differentiated to mature ones (data not shown). From these observations, we are tempting to suggest that the adjuvant activity shown by acemannan is due at least in part to the differentiation-inducing activity on immature DCs.

The mechanism(s) for the differentiation-inducing activity of acemannan requires further investigation. However, we observed that oversulfation of acemannan, a procedure that modifies the surface structure of acemannan, abrogates the differentiation-inducing activity as well as the mitogenic activity almost completely. DCs have been known to express mannose receptors [18–20]. Because acemannan is a polymer of acetylated mannose, it is reasonable to hypothesize that acemannan binds to DCs through mannose receptors, and chemical sulfation inhibits such specific binding. The possibility of acemannan binding DC mannose receptors was explored by adding excess amount of mannose into cell culture. When mannose was added to the culture at the same time with acemannan, acemannan-induced phenotypic and functional maturation of immature DCs was not suppressed significantly. Thus, it appears that acemannan interacts with cell surface binding sites on immature DCs that are not shared with mannose. The effect of mannose, however, was pronounced in suppressing the mitogenic activity of acemannan, suggesting that the mitogenic activity of acemannan is at least partially through its action on mannose receptors.

In summary, we showed evidences that acemannan, a major carbohydrate fraction of *Aloe vera* gel, could induce functional and phenotypic maturation of immature DCs in vitro. In addition, we showed evidences that chemical sulfation of acemannan abrogates the differentiation-inducing activity almost completely. Furthermore, we suggest that mannose receptors may be involved in some of the biological activities of acemannan. These observations may

have important implications in understanding the antiviral and antitumoral activities of acemannan.

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