Dendritic Cells Stimulated with a Bacterial Product, OK-432, Efficiently Induce Cytotoxic T Lymphocytes Specific to Tumor Rejection Peptide

Saori Nakahara, Takuya Tsunoda, Toshiyuki Baba, Shinichi Asabe, and Hideaki Tahara

Department of Surgery and Bioengineering, Institute of Medical Science, The University of Tokyo, Tokyo 108-8639, Japan [S. N., T. T., T. B., H. T.], and Immunology Division, Jichi Medical School, Tochigi-Ken 329-0049, Japan [S. A.]

ABSTRACT

Dendritic cells (DCs) are potent antigen-presenting cells, which have recently been applied for cancer immunotherapy using epitope peptides. Accumulating results of the clinical trials of such a strategy suggest that maturity of the applied DCs has a significant impact on the outcome of the vaccination. Here we examined the effects of penicillin-killed Streptococcus pyogenes (OK-432) on DC maturation and functions including induction of CTLs. DCs generated from peripheral blood using granulocyte macrophage colony-stimulating factor and interleukin (IL)-4 showed immunophenotypes consistent with immature DCs (iDCs). These iDCs were further incubated with medium alone, tumor necrosis factor α, lipopolysaccharide, or OK-432. The immunophenotypical analysis showed DCs stimulated with OK-432 (OK-DCs) possessed significantly higher expression of CD83 compared with unstimulated DCs. Furthermore, OK-DCs showed significantly higher production of IL-12 and IFN-γ compared with DCs with other stimulations. These results indicate that OK-432 stimulates iDCs to have a mature phenotype and to produce a significant amount of T-helper 1-type cytokines. To examine the potency of OK-DCs on the induction of specific CTLs, the tumor rejection peptide derived from carcinoembryonic antigen was used as a model antigen. The HLA-tetramer assay showed that potent CTL was induced with OK-DCs at high frequency. These results indicate that OK-432 efficiently stimulates DCs without interfering with the presentation of pulsed peptide. Furthermore, OK-432 does not activate nuclear factor-κB through Toll-like receptor 2 or Toll-like receptor 4 in the indicator cell system; however, it induces IL-12 production through the β2 integrin system on DCs. These results strongly suggest that OK-432 could be applied to develop an efficient cancer vaccine using DCs pulsed with tumor rejection peptides.

INTRODUCTION

DCs are considered to be the most potent antigen-presenting cells, and they regulate T-cell responses to microbial pathogens, virus-infected cells, dead cells, and tumor cells. Recent development of in vitro manipulation of DCs enables us to perform clinical studies of DC-based cancer vaccines for patients with various diseases including colon cancer, gastric cancer, B-cell lymphoma, breast cancer, lung cancer, esophageal cancer, and malignant melanoma (1–15). In such studies of DC-based cancer vaccines, various strategies have been applied to have DCs present tumor rejection antigens. These strategies include cocultivation of DCs with apoptotic necrotic tumor cells, tumor lysates, synthetic epitope peptide, protein on DCs, and genes encoding tumor rejection antigens. In virtually all these strategies, the nature of the resulting immune response in the treated patients appears to correlate with the characteristics of the DCs used. In this study, we investigated the means to generate DCs that can efficiently induce CTLs specific to the tumor antigen peptides.

When synthetic peptides are used as antigen presented on class I complex on DCs, a potent immune response could be efficiently induced only with mature DCs, which express abundant MHC class I and class II costimulatory molecules including CD80 and CD86 and secrete cytokines including IL-12. Administration of peptide-pulsed DCs cannot induce full stimulation but conversely induces antigen-specific T-cell inhibition (16). Thus, mature DCs should be used when DCs pulsed with synthetic peptides are used for the treatment of cancers.

Maturation of DCs can be influenced by a variety of cytokines including IL-1, GM-CSF, and TNF-α (17, 18). To optimally induce DC maturation, especially in humans, these cytokines have been used at various timings, concentrations, and durations and in various combinations. TNF-α might be one of the most commonly used cytokines for this purpose. However, it has recently been reported that TNF-α alone cannot induce full maturation of DCs. Thus, it is now evident that multiple cytokines should be used for this purpose (19–21). In addition to these cytokines, bacteria (22) and related molecules including LPS (cell wall component of Gram-negative bacteria; Ref. 23) have been shown to stimulate DC maturation.

In this study, we examined the possibility of using one of these molecules for DC maturation. OK-432, a penicillin-killed and lyophilized preparation of a low-virulence strain (Su) of Streptococcus pyogenes (group A), has been clinically available in Japan for more than 20 years (24). Although the therapeutic benefit of OK-432 administration has been proved in a limited cohort of cancer patients, OK-432 has been shown to stimulate multiple cell types including macrophages to produce various cytokines, such as IFN-γ, TNF-α, IL-6, IL-8, IL-10, IL-12, and IL-18, from these cells (25). From these sets of information, we hypothesized that OK-432 might modulate DC maturation. The effects of OK-432 on phenotypes and functions of DCs were examined in comparison with those of TNF-α and LPS. This study shows that DCs incubated with OK-432 are phenotypically compatible with mature DCs, abundantly produce Th1-type cytokines, and can efficiently induce potent CTLs specific to the epitope peptide of tumor-associated antigen pulsed on DCs. Furthermore, the mechanism of OK-432 stimulation on DCs was examined focusing on TRL2, TLR4, and β2 integrin, which have been known as receptors for various microbial antigens.

MATERIALS AND METHODS

Media, Cytokines, Antibodies, and Reagents. For DC generation and CTL induction, AIM-V (Life Technologies, Inc., Gaithersburg, MD) containing 2% autologous plasma was used. For culturing Ba/F3 and its transfectants, RPMI 1640 supplemented with 10% fetal bovine serum and 1000 units/ml IL-3 was used. Human recombinant GM-CSF and human recombinant IL-4 were generous gifts from Kirin Brewery Co. and Ono Pharmaceutical Co, respectively. Human recombinant TNF-α was purchased from Genzyme/Technoe. LPS (Escherichia coli 055:B5), PGN, and β2-microglobulin were purchased from Sigma (St. Louis, MO). OK-432 was provided by Chugai Pharmaceutical.
The following antibodies were used for flow cytometry: (a) FITC-conjugated mouse antihuman IgG2a isotype control (BD Pharmingen); (b) PE-conjugated mouse antihuman IgG1 isotype control (BD Pharmingen); (c) FITC-conjugated mouse antihuman CD1a (DAKO, Carpinteria, CA); (d) PE-conjugated mouse antihuman CD14 (BD Pharmingen); (e) FITC-conjugated mouse antihuman CD14 (BD Pharmingen); (f) PE-conjugated mouse antihuman CD80; (g) PE-conjugated mouse antihuman CD40 (Immunootech, Marseilles, France); (h) FITC-conjugated mouse antihuman CD86 (BD Pharmingen); (i) PE-conjugated mouse antihuman CD83 (Coulter Immunology, Miami, FL); (j) FITC-conjugated mouse antihuman HLA-DR (BD Pharmingen); (k) PE-conjugated mouse antihuman HLA-A, HLA-B, and HLA-C (DAKO); and (l) FITC-conjugated mouse antihuman CD8 (BD Pharmingen). Mouse IgG1 isotype antibody, mouse antihuman CD11b antibody, mouse antihuman CD11c antibody, mouse antihuman CD14 antibody, and mouse antihuman CD18 antibody were purchased BD Pharmingen.

**Synthetic Peptide.** CE3 peptide was synthesized and purified by Takara Shuzo Co. Ltd. using the standard solid-phase method and purified by high-performance liquid chromatography. The purity (>95%) and identity of peptide were confirmed by mass spectrometry analysis.

**Preparation of DCs.** Highly purified DCs were obtained using previously described methods (26), with minor modifications. In brief, peripheral blood mononuclear cells were isolated from normal healthy volunteers in heparinized syringes, and PBMCs were isolated by sedimentation over Ficoll-Hypaque (Amersham Pharmacia Biotech, Piscataway, NJ). After rinsing three times with PBS, the cells were isolated again with Histopaque 1083 (Sigma), rinsed three times with PBS, placed into 10-cm culture plates at 2.5 × 10⁵ cells/plate, and cultured for 30 min at 37°C. The nonadherent cells in the plate were removed, and the remaining adherent cells were cultured overnight in AIM-V containing 2% autologous plasma. The following day, nonadherent cells were removed, and the medium was changed to AIM-V containing 2% autologous plasma supplemented with 1000 units/ml GM-CSF and 1000 units/ml IL-4. After 5–7 days, nonadherent cells were harvested and used for subsequent experiments.

**Flow Cytometric Analysis.** Cells (1 × 10⁷) were incubated with specific antibodies or isotype-matched control mouse IgG (Becton Dickinson, San Diego, CA) conjugated with FITC or PE in PBS containing 0.2% BSA for 30 min at 4°C, rinsed twice, resuspended with 0.1% paraformaldehyde, and examined using a fluorescence-activated cell sorter (FACS Calibur) with CellQuest software (Becton Dickinson, Mountain View, CA).

**Cytokine Production Assay.** iDCs were plated on a 96-well round-bottomed plate (2 × 10⁵ cells/ml) and cultured for 6–72 h in medium (200 µl/well) containing TNF-α (100 ng/ml), LPS (100 ng/ml), or OK-432 (10 µg/ml) or in medium only, with or without various antibodies. After the incubation, supernatant was collected and stored at −80°C until the assay. The concentration of IL-12 (p40 and p70), IFN-γ, IL-4, IL-5, and IL-10 was measured with corresponding human immunoassay kits (Endogen). Each assay was performed on duplicate samples.

**CTL Induction using Peptide-pulsed DCs.** DCs were cultured under various conditions, pulsed with antigenic peptide, and used as antigen-presenting cells to stimulate autologous CD8⁺ T cells. The 9-mer peptide CE3 (TYACFVSNL), derived from CEA, was used as a model antigen for CTL induction using these DCs. CEA3 was identified as a HLA-A*2402-restricted epitope peptide that can induce CTLs specific to CEA, tumor cells in vitro and in vivo (27, 28). Generated DCs were pulsed with 20 µg/ml peptide in the presence of 3 µg/ml β2-microglobulin for 4 h at room temperature. The peptide-loaded DCs were irradiated (55 Gy) and cultured in a 6-well plate mixed with CD8⁺ T cells obtained by positive selection with Dynabeads M-450 CD8 and Detachabead (both from Dynal, Lake Success, NY) in AIM-V containing 2% autologous plasma. There were 1.5 × 10⁴ DCs and 3 × 10⁶ CD8⁺ T cells in 6 ml of medium at the beginning of the culture, and irradiated DCs (1.5 × 10⁶ cells) were added into the culture on day 7 and day 14 for restimulation (29).

**Cytotoxicity Assay.** Cytolytic activity was determined using a standard 4-h ⁵¹Cr release assay. TISI cells, EBV-immortalized human B cells that express HLA-A*2402 but not endogenous CEA, were kindly provided by Takara Shuzo Co. Ltd., incubated with 10 µg/ml CE3 peptide overnight at 37°C, and used as a model target (TISI-CE3). A total of 5 × 10⁵ TISI-CE3 and unpulsed TISI cells were labeled with 100 µCi of ⁵¹Cr-labeled sodium chromate for 1 h at 37°C. Labeled target cells (1 × 10⁵ cells/well) and various numbers of effector cells were plated in a 96-well plate at a total volume of 200 µl. After incubation at 37°C for 4 h, 100 µl of supernatant were collected from each well, and the percentage of specific lysis was determined according to the formula below.

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\text{Experimental release} = \left(1 - \frac{\text{Spontaneous release}}{\text{Maximal release}}\right) \times 100
\]

**TLR Indicator Cell Lines and Luciferase Assay.** TLR indicator cell lines are generous gifts from Drs. Kensuke Miyake and Sachiko Akashi. Mouse TLR2 gene, mouse TLR4 and mouse MD2 genes, or human TLR4 and human MD2 genes were transfected into Ba/F3 cells, an IL-3-dependent mouse myeloid cell line (33), by electroporation. These transfectants were all cotransfected with pSG5Luc, which is a reporter construct of NF-κB activation (34). These stable transfectants were plated in a 96-well plate (1 × 10⁵ cells/well), and various concentrations of PGN, LPS, or OK-432 were incubated with the cells at 37°C for 3 h to examine their receptor usage. The cells were lysed with lysis buffer (Sigma) after incubation, and 10 µl of supernatant were harvested. The supernatant were developed with 50 µl of luciferase substrate and applied to luminometer.

**Statistical Analysis.** Every experiment was performed at least three times to confirm reproducibility of the results, and results representative of the similar results are shown. Student’s t test was used to examine the significance of the data, when applicable. The difference was considered to be statistically significant when P was <0.05.

**RESULTS**

**OK-432 Induces DC Maturation.** Characteristics of the DCs were examined after culturing for 5–7 days under the condition of DC generation described in “Materials and Methods.” Under microscopic observation, >90% of harvested cells showed the typical morphological features of DCs. In every experiment, FACS analysis showed that >90% of the prepared cells were CD11c⁺/CD80⁺/MHC class II⁺/MHC class II⁺, and 5–5% were CD14⁺ or CD3⁺. The populations of CD80⁺, CD86⁺, and CD83⁺ cells in these cells were 8.2 ± 7.3%, 51.5 ± 15.1%, and 2.7 ± 0.7%, respectively. These characteristics showed that the phenotypes of the cells generated under this culture condition were consistent with those of iDCs.

On day 7, these iDCs were harvested, divided into four groups, and cultured in medium alone (group A), medium containing 100 ng/ml TNF-α (group B; TNF-α-DCs), medium containing 100 ng/ml LPS (group C; LPS-DCs), or medium containing 10 µg/ml OK-432 (group D; OK-DCs). The concentration of each reagent was selected to induce the highest expression of CD83 after testing the following concentrations: TNF-α, 3–300 ng/ml; LPS, 0.01–10 µg/ml; and OK-432, 0.1–30 µg/ml (data not shown). After incubation at 37°C for 72 h, cells were harvested and analyzed for the expression of cell surface antigens using FACS analysis. The expression of CD80, CD86, and CD83 in each group was as follows: group A, 16.5%, 8.2%, and 7.3%; group B, 53.2%, 99.7%, and 85.9%; group C, 65.7%, 99.6%, and 84.7%; and group D, 58.8%, 99.7%, and 61.0% (Fig. 1). The expression of CD83, which is expressed on mature DCs (35–38), was up-regulated not only by TNF-α or LPS, but also by

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OK-432 stimulation also induced high-level IFN-γ secretion from DCs at a much higher level than stimulation with LPS, another microbial stimulant. To explore the mechanism that causes this difference, we examined the involvement of TLRs, which had recently been identified as receptors for microbial pathogens within the human TLRs. TLR2 is used by some Gram-positive whole bacterial bodies and their derivatives including PGN and lipoteichoic acid, while TLR4 uses CD14 and TLR2 or TLR4, forming a complex with MD2, is used by LPS (36, 37).

**OK-432 Does Not Bind to TLR2 or TLR4.** Our results showed that stimulation with OK-432 could induce the secretion of Th1-type cytokines at a much higher level than stimulation with LPS, another microbial stimulant. To explore the mechanism that causes this difference, we examined the involvement of TLRs, which had recently been identified as receptors for microbial pathogens within the human TLRs. TLR2 is used by some Gram-positive whole bacterial bodies and their derivatives including PGN and lipoteichoic acid, while TLR4 uses CD14 and TLR2 or TLR4, forming a complex with MD2, is used by LPS (36, 37).

![Graph](image)

**Fig. 2.** Secretion of Th1-type cytokines induced with TNF-α, LPS, or OK-432. On day 7, iDCs were harvested, washed twice with PBS, and plated on a 96-well round-bottomed plate (4 × 10^5 cells/well) with culture medium consisting of medium alone, 100 ng/ml TNF-α, 100 ng/ml LPS, or 10 μg/ml OK-432 at a final volume of 200 μl in each well. After incubation at 37°C for 72 h, supernatant was collected and analyzed for cytokine concentration using ELISA. The OK-432 stimulation induced IL-12 secretion from DCs at a high level (40.7 ± 3.1 ng/ml) that was significantly higher than that of the control (0.3 ± 0.3 ng/ml; P < 0.0001) or those induced with TNF-α (1.7 ± 2.1 ng/ml; P < 0.001) or LPS (5.6 ± 4.7 ng/ml; P < 0.005; Fig. 2a). Furthermore, OK-432 stimulation also induced IFN-γ secretion from DCs at a high level (197.6 ± 272.6 pg/ml) that was markedly and significantly higher than that of the control (<25.6 pg/ml; P < 0.0001) or those induced with TNF-α (47.7 ± 81.3 pg/ml; P < 0.01) or LPS (248.6 ± 263.1 pg/ml; P < 0.01; Fig. 2b). On the other hand, the concentrations of IL-4, IL-5, or IL-10, all of which are considered to be Th2-type cytokines, were below the detection limits of ELISA kits up to 72 h after the stimulation (data not shown). These results suggest that DCs stimulated with OK-432 (OK-DCs) preferentially secrete Th1-type cytokines at a high level.

**Peptide-pulsed OK-DCs Strongly Induce CTLs Specific to the Pulsed Peptide.** Although OK-DCs have a mature phenotype expressing Th1-type cytokines, it was not clear whether peptide-pulsed DCs could efficiently induce potent CTLs specific to the pulsed peptide.

To answer this question, four groups of DCs were prepared as described in the previous section. DCs in each group were pulsed with 20 μg/ml CE3 peptide in the presence of 3 μg/ml β2-microglobulin for 4 h at room temperature. The DCs loaded with CE3 peptide were irradiated at 55 Gy and mixed with naive CD8^+ T cells that were obtained by positive selection using Dynabeads M-450 CD8 and Detachabead from the PBMCs at a ratio of 1:20 (1.5 × 10^5 DCs and 3 × 10^6 CD8^+ T cells) in 6 ml of AIM-V medium containing 2% autologous plasma using a 6-well plate. On days 7 and 14, restimulation was performed using 1.5 × 10^5 DCs pulsed with CE3 peptide. The independent experiment was performed to induce CTL from five healthy donors, and representative data among similar results are shown in Fig. 3A. Unstimulated iDCs failed to induce potent CTLs specific to the target cells pulsed with CE3. Although DCs stimulated with either TNF-α or LPS did not induce potent CTLs, OK-DCs induced markedly potent CTLs specific to the target cells pulsed with CE3 peptide. The characteristics of these CTLs in each group were also examined using tetramer assay. CTLs induced with OK-DCs stained positive for both CD8 and CE3-tetramer at 9.6%, which was much higher than those of CTLs induced with unstimulated DCs (1.8%), TNF-α-DCs (3.4%), or LPS-DCs (3.1%; Fig. 3B). In three of five experiments, tetramer assays were done chronologically after every stimulation. The frequencies of CD8^+CE3-tetramer^+ cells gradually increased after multiple stimulations with OK-432. The frequencies of such double-positive cells were always highest in CTLs induced with OK-DCs (Fig. 3C). These results strongly suggest that peptide-loaded OK-DCs can effectively present pulsed epitope peptide of a tumor-associated antigen to T cells and induce potent CTLs specific to the target cells presenting such an antigen.

**OK-432 Does Not Bind to TLR2 or TLR4.** Our results showed that stimulation with OK-432 could induce the secretion of Th1-type cytokines at a much higher level than stimulation with LPS, another microbial stimulant. To explore the mechanism that causes this difference, we examined the involvement of TLRs, which had recently been identified as receptors for microbial pathogens within the human TLRs. TLR2 is used by some Gram-positive whole bacterial bodies and their derivatives including PGN and lipoteichoic acid (35, 39, 40), and TLR4, forming a complex with MD2, is used by LPS (36, 37).
Thus, we hypothesized that OK-432 could use TLR2 or TLR4 if TLRs are involved in OK-432 stimulation.

To examine the usage of TLR2 and TLR4, respective indicator cell lines were used. As shown in Fig. 4, top right panel, OK-432 did not induce luciferase activity of Ba/F3 mouse TLR2 through NF-κB even at 10 μg/ml, although PGN induced significantly high luciferase activity at a much lower concentration of 10 ng/ml. Furthermore, OK-432 did not induce luciferase activity of Ba/F3 cells expressing mouse or human TLR4 with MD2 either, although LPS did so even at a lower concentration (10 ng/ml, Fig. 4, bottom panels). These results suggest that neither TLR2 nor TLR4 is involved in the process of OK-432 stimulation in this assay system.

β₂ Integrins Are Involved in the Signaling Pathway of OK-432.

To investigate the receptor mechanism of OK-432 stimulation, involvement of β₂ integrin, which is another microbial receptor, was examined. To block the cross-link of OK-432 and each component of β₂ integrin receptor systems, anti-CD11b antibody, anti-CD11c antibody, or anti-CD18 antibody was added at a final concentration of 15 μg/ml for 30 min at 37°C before incubating iDCs with 10 μg/ml OK-432 for 6 h at 37°C (Fig. 5). In theory, cross-linking of OK-432 to CR3 (heterodimer of CD11b and CD18) and CR4 (heterodimer of CD11c and CD18) could be blocked with these antibodies.

Although the production of IL-12 induced by OK-432 was not significantly inhibited with mouse isotype IgG antibody or anti-
CD11b antibody, IL-12 production was significantly suppressed with anti-CD11c antibody (P < 0.01) and anti-CD18 antibody (P < 0.05) when compared to IL-12 production without any antibody. These data suggest that β2 integrins are involved, at least partially, in the process of OK-432 stimulation.

**DISCUSSION**

In this study, we examined the effects of OK-432 on iDCs derived from human PBMCs. OK-DCs had significantly up-regulated CD83 (P < 0.001) and produce a significant amount of Th1-type cytokines IL-12 and IFN-γ. The production levels of both cytokines were significantly higher than those of TNF-α-DCs or LPS-DCs. However, Th2-type cytokines including IL-4, IL-5, and IL-10 were not detected within 72 h after OK-432 stimulation. These data suggest that OK-DCs might have favorable characteristics to induce CTLs specific to antigen peptide. To directly address this question, DCs pulsed with CE3 peptide were used for CTL induction in vitro. In this experiment, OK-DCs were shown to induce the most potent CTLs specific to CE3 peptide when compared with those induced by iDCs, TNF-α-DCs, or LPS-DCs. Furthermore, the β2 integrin system, but not TLR2 or TLR4, appeared to be involved in OK-432 stimulation.

Results of recent clinical studies strongly suggest that mature DCs should be used in the cancer vaccine protocols using synthetic peptides as antigens presented on MHC class I. Mature DCs efficiently induce tumor-specific CTLs with their characteristics including up-regulated MHC class I and costimulatory molecules such as CD80 and CD86. Mature DCs can also produce Th1-type cytokine such as IL-12 for T-cell stimulation upon certain stimulation (41–43). Furthermore, mature DCs have a high migratory activity and express CCR7, a receptor for chemokines produced constitutively in lymphoid tissues (44). To the contrary, it was reported recently that peptide-loaded iDCs led to the specific inhibition of the effector function of peptide-specific CD8+ T cells, in contrast to the findings using mature DCs (16). TNF-α is well known as one of stimulants that switch DCs to an immunostimulatory mode, but TNF-α alone is not potent enough to lead DCs to fully mature (19). Thus, MCM has been used to induce final maturation of DCs by some investigators (19, 20). Although MCM has some advantages, the yield and quality of DCs generated with different MCMs varies substantially. To overcome these disadvantages, multiple investigators have been trying to identify defined cytokine mixture (45). Jonuleit et al. (21) reported that a mixture of TNF-α, IL-1β, and IL-6 could induce DC maturation as potently as MCM. They also reported that addition of prostaglandin E2 to this cytokine mixture further enhanced the yield, maturation, and migratory and immunostimulatory capacity of the generated DCs.

We investigated the effects of OK-432, a bacterial product for which safety in clinical use has been established, on DC maturation. OK-DCs have morphology and phenotype compatible with mature DCs and abundantly secrete Th1-type cytokines including IL-12 and IFN-γ at a level significantly higher than those of iDCs, TNF-α-DCs, or LPS-DCs. The amount of IL-12 secreted from OK-DCs was much higher than that secreted from the DCs stimulated with the reported mixture of TNF-α, IL-1β, IL-6, and prostaglandin E2 (data not shown). Furthermore, OK-DCs efficiently induce CTLs specific to the pulsed CE3 peptide. Because OK-432 itself has its own antigenic epitopes that could be abundantly expressed and fully occupy class I molecules, there is a concern that the presentation of other antigenic epitopes could be inhibited. The HLA tetramer binding assay showed that the frequency of the CD8+ T cells binding to HLA-A*2402/CE3 was significantly high in the T cells stimulated with OK-DCs. The 51Cr release assay also showed the highest cytolytic activity for the T cells stimulated with OK-DCs. These results strongly suggest that
OK-432 can lead the iDCs to immunopotent mature DCs, which can induce CTLs specific to the pulsed antigen, and that peptide presentation is not interfered with by the antigens derived from OK-432 itself. We then investigated the mechanism of OK-432 stimulation. First, the involvement of TLRs, which have recently been shown to regulate the interface of innate and adaptive immunities, was examined. Toll, *Drosophila* receptor, was originally identified as a receptor for protective immunity of fungal infection in flies (45), and a number of immunologically relevant homologues of Toll have been discovered since then in organisms including plants, insects, and mammals. In the human, nine TLR homologues have been identified to date (46–50), and each receptor recognizes specific pathogen. TLR2 is involved in the signaling pathways between OK-432 and LPS might be associated with dendritic cells presenting epitopes derived from the melanoma-associated antigen MART-1 and gp100. J. Immunother., 23: 487–498, 2000.


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