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Effects of adenoviral gene transfer of mutated IκBα, a novel inhibitor of NF-κB, on human monocyte-derived dendritic cells[1]

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Key words

dendritic cells; nuclear factor κB; IκBα mutant; immunotherapy; asthma

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Abstract

Aim: To investigate the effects of adenoviral gene transfer of IκBα mutant (IκBαM), a novel inhibitor of nuclear factor κB (NF-κB), on apoptosis, phenotype and function of human monocyte-derived dendritic cells (DC). **Methods:** Monocytes, cocultured with granulocyte/macrophage colony-stimulating factor (GM-CSF; 900 ng/mL) and interleukin (IL)-4 (300 ng/mL) for 5 d, followed by stimulation with lipopolysaccharide (LPS; 100 ng/mL) for 2 d differentiated into mature DC. Monocytes were either left untransfected or were transfected with AdIκBαM or AdLacZ. The transcription and expression of the IκBαM gene, and the inhibitory effect of IκBαM on tumor necrosis factor (TNF)-α-induced NF-κB activation in mature DC were detected by polymerase chain reaction (PCR), reverse transcription-polymerase chain reaction (RT-PCR), Western blot analysis, and electrophoretic mobility shift assays, respectively. The phenotype, apoptosis, IL-12 secretion level of DC, and ability to stimulate the proliferation of T cells were determined by flow cytometry, enzyme-linked immunosorbent assay and mixed leukocyte reaction. **Results:** PCR and RT-PCR were used to detect a unique 801 bp band in AdIκBαM-transfected mature DC, and also a dose- and time-dependent expression of the IκBαM gene, which peaked at a multiplicity of infection of 100 pfu/cell and at 48 h. Furthermore, AdIκBαM significantly suppressed the TNF-α-induced NF-κB activation, augmented apoptosis, downregulated CD80, CD83, and CD86 surface molecules, IL-12 secretion levels and the ability to stimulate the proliferation of T cells in mature DC. **Conclusion:** AdIκBαM effectively transfected and potently inhibited NF-κB activation in monocyte-derived mature DC. Overexpression of the IκBαM gene in mature DC may contribute to T-cell immunosuppression through induction of DC apoptosis and downregulation of B7 molecules, providing a potential strategy for future DC-based immunotherapy of asthma.

Introduction

Atopic asthma, characteristic of eosinophilic airway inflammation, is now regarded as a T-helper 2 (Th2) cell-mediated disorder, which is under the control of dendritic cells (DC)[1]. Studies in rodents and humans have revealed the presence of an extensive network of DC in airways[2,3]. Upon maturation *in vivo* or *in vitro*, DC become less phagocytic and more potent antigen-presenting cells (APC), expressing high levels of major histocompatibility complex (MHC) class II and costimulatory molecules as well as proinflammatory cytokines. As the most potent professional APC identified, DC play a central role in inducing the primary immune response, priming the naive T cells, initiating the immune tolerance, and regulating the types of T cell responses[4].

Nuclear factor κB (NF-κB), which is typical of p50/p65 heterodimers, is sequestered as inactive trimers in the cytoplasm of quiescent cells through interaction with IκBα, the most important member of the inhibitors of NF-κB (IκB)

family. Multiple signals converge on the common signaling pathway for NF-κB activation upon sequential phosphorylation of IκBα at the Ser 32/36 residues of the N-terminal domain, and subsequent proteolytic degradation[5]. Recent data suggest that NF-κB overactivation may be the basis for increased expression of myriad inflammatory genes and for the perpetuation of chronic airway inflammation in asthma[6,7]. More recently, we have cloned the 801 bp Chinese IκBα mutant (IκBαM) gene (203–1003 bp) encoding 267 amino acids from human placenta, a novel nondegradable superrepressor of NF-κB, by site-directed deletion of the N-terminal phosphorylation sites of Ser 32/36, and constructed a replication-deficient recombinant adenovirus vector AdIκBαM[8]. These mutations prevent IκBαM phosphorylation, retaining NF-κB in its inactivated cytosolic location complexed with IκBαM. DC maturation and cytokine production are NF-κB-dependent, thus, we investigated the effects of adenoviral gene transfer of IκBαM on apoptosis, phenotype and function of DC derived from human monocytes to explore a possible approach for future DC-based immunotherapy of asthma.

Materials and methods

Reagents Recombinant human granulocyte/macrophage colony-stimulating factor (GM-CSF), interleukin 4 (IL-4), and tumor necrosis factor α (TNF-α) were generously provided by Dr Guang-yong PENG (Baylor College of Medicine, Dallas, USA). Lipopolysaccharide (LPS) was purchased from Sigma Chemical Co (St Louis, MO, USA). Fluorescein isothiocyanate (FITC)-conjugated mouse anti-human CD14, HLA-DR, CD83 and CD80 were bought from Serotec (UK), and phycoerythrin (PE)-conjugated mouse anti-human CD86 were bought from Diaclone (USA). Taq DNA polymerase and T4 DNA ligase were obtained from Gibco BRL (USA). [γ-^{32}P] adenosine triphosphate and [^{3}H] thymidine (TdR) were provided by Beijing Furui Biotechnology (China).

Vectors The serotype 5-, E1-, and E3-defective adenoviruses expressing *Escherichia coli* β-galactosidase (AdLacZ) and human IκBαM (AdIκBαM) were constructed as described previously[8]. Remarkably, the molecular weight of novel IκBαM was deduced to be 30 kDa. The generated titers of AdLacZ and AdIκBαM were approximately 1.0×10^{10} and 4.0×10^{9} pfu/mL, respectively.

Cell culture Blood buffy coat from healthy donors (Jiangsu Institute of Hematology) was centrifuged on Ficoll-Paque (Pharmacia, Sweden) to acquire human peripheral blood mononuclear cells (PBMC). Monocytes were derived from PBMC depleted of NK, B-, and T cells with anti-CD16, anti-CD19 and anti-CD3, as well as goat anti-mouse Ig-conjugated magnetic beads (Miltenyi Biotec, USA) as previously described[9]. These were plated (1×10^{7} cells/3 mL per well) into 6-well plates in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS; Gibco BRL, USA). Cells were cultured at 37 °C in 5% CO_2 in medium supplemented with GM-CSF (900 ng/mL) and IL-4 (300 ng/mL). Cultures were fed every 2 d with half of the culture volume of full doses of cytokines. On d 5 cells were stimulated with LPS (100 ng/mL) to induce maturation, and the suspended cells were harvested on d 7.

Electron microscopy On d 7 cells were washed once in phosphate-buffered saline (PBS), resuspended, and fixed overnight in 2% paraformaldehyde and 2.5% glutaraldehyde, followed by fixation for 1 h in 1% cacodylate-buffered osmium tetraoxide. After dehydration in a series of ethanol and propylene oxide solutions, the cells were embedded in epoxy resin, ultrathinly sectioned, stained with uranyl acetate and lead citrate, and examined under a scanning electron microscope (SEM; SX-40; Akashi, Japan). Additionally, the prepared cells were routinely stained and examined under a transmission electron microscope (TEM; JEM-1010; Jeol, Japan).

Gene transfer Adenoviral transfections were performed in 60 mm plates with mature DC at 5×10^{5} cells/plate on d 7. For adenoviral transfections, medium was removed from each plate and replaced with 2 mL of medium containing either AdIκBαM at multiplicity of infection (MOI) of 25, 50, or 100 pfu/cell, or AdLacZ at an MOI of 50 pfu/cell for 4 h, followed by incubation in fresh RPMI-1640 medium supplemented with 10% FCS at 37 °C and in 5% CO_2 for an additional 24 h and 48 h, respectively. The untransfected mature DC were used as normal controls.

PCR and RT-PCR DNA and RNA were extracted from mature DC, either untransfected or transfected with AdIκBαM and AdLacZ, using Wizard plasmid isolation and Trizol kits (Promega, USA), according to the manufacturer's instructions, and the RNA was reverse-transcribed into cDNA for templates. Primers were designed using Primer Express software and were as follows: forward primer 5'-CCTCTAGAATGAAAGACGAGGAGTACGAG-3' and reverse primer 5'-TGGTACCTCACAGCTCGTCCTCTGTGAACTCCGTG-3'. The reaction started with an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 54 °C for 1 min and extension at 72 °C for 1 min and additional extension of 10 min, after which 5 μL of each product was quantified by 1% agarose gel electrophoresis.

Western blot analysis After 24 or 48 h of overexpression

of the genes of interest, cytosolic proteins were prepared as described elsewhere[10] and quantitated by using the Bradford assay. Equivalent amounts of denatured protein (100 μg) were separated on 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel, and electrophoretically transferred to nitrocellulose membranes. Nonspecific binding to the membrane was blocked with 5% nonfat dry milk in PBS-Tween overnight at 4 °C. Blots were washed in PBS-Tween, and then probed with anti-human IκBα antibody (1:1000 dilution) and horseradish peroxidase-conjugated anti-goat IgG (1:5000 dilution, Santa Cruz Biotechnology, USA) for 2 h at room temperature. Immunoblots were visualized with an enhanced chemiluminescence detection kit (Amersham Life Sciences, USA).

Electrophoretic mobility shift assay After 48 h overexpression of target genes, DC were harvested upon stimulation with 10 ng/mL TNF-α for 30 min. Preparation of nuclear extracts and the electrophoretic mobility shift assay (EMSA) were carried out as described previously[11]. The NF-κB oligonucleotide(5'-AGTTGAGGGGACTTTCCCA-GGC-3')contained a consensus NF-κB motif (underlined), and the mutant NF-κB oligonucleotide (5'-AGTTGAGGGT-CCTTTCC-CAGGC-3'; Promega, USA) was used as a mutant competitor. Five micrograms of nuclear extracts were incubated with 30 fmol of [^{32}P]-labeled oligonucleotide by T4 polynucleotide kinase for 30 min at room temperature. DNA-protein complexes were electrophoretically resolved on 5% nondenatured polyacrylamide gel, which was visualized and quantitated by PhosphorImager (Molecular Dynamics) using ImageQuant software (Amersham Life Sciences, USA). For competition assays, a 100-fold excess of unlabeled NF-κB or mutant NF-κB oligonucleotide was added to the nuclear extracts from TNF-α-stimulated cells 10 min before exposure to the [^{32}P]-labeled probe.

Flow cytometry DC that were either untransfected or transfected with AdIκBαM or AdLacZ were incubated at a density of 1×10^5 cells/100 μL with 10 μL FITC- or PE-conjugated monoclonal antibodies for 30 min at room temperature. The cells were washed once in PBS or fixed with 1% paraformaldehyde, then the surface markers of mature DC were assessed by FACScan (Becton Dickinson, USA). In addition, apoptosis of mature DC was immediately analyzed by FACScan after staining with 5 μL annexin V (AV)-FITC and 5 μL propidium iodide (PI) for 20 min at 4 °C. The total cell population consisted of DC that were AV single positive (apoptotic), PI single positive (necrotic), AV and PI double positive (apo-necrotic), or AV and PI double negative (live).

Enzyme-linked immunosorbent assay Supernatants from mature DC were harvested 48 h after transfection with AdIκBαM and AdLacZ. Levels of IL-12 (p70) were determined using a sandwich enzyme-linked immunosorbent assay (ELISA) kit (PharMingen, USA) in compliance with the manufacturer's instructions.

Mixed leukocyte reaction Allogeneic T cells were obtained from nylon wool nonadherent PBMC by negative selection, with a purity of more than 95%[12]. T cells were cultured in triplicate in 96-well flat-bottomed plates at 2×10^5 cells/well, to which nonautologous mature DC, either untransfected or transfected with AdIκBαM and AdLacZ, were added at 2×10^3, 4×10^3, 10×10^3, or 20×10^3 cells/well. Plates were incubated at 37 °C and 5% CO_2 for 96 h, and pulsed with 0.5 μCi/well of [^{3}H]thymidine for the final 16 h before harvesting on filters for scintillation counting. Counts per minute (cpm) of triplicate wells were deduced by subtracting the background cpm of the medium alone.

Statistical analysis Data were expressed as mean±SD, and assessed by one-way analysis of variance (ANOVA) and paired Student's *t*-tests with SPSS 11.0 software. Statistical difference was assumed at $P<0.05$.

Results

Morphology of mature DC In culture with GM-CSF and IL-4 for 5 d, monocytes differentiated into mature DC after stimulation with LPS for 2 d. The mature DC showed prominent dendritic and veiled projections (>10 μm) on d 7 under the SEM (Figure 1A), and displayed plentiful surface dendrites and cytosolic vesicles mostly of endocytic type, but a relatively immature Golgi zone under the TEM (Figure 1B).

Detection of IκBαM gene in mature DC Electrophoretic analysis of both PCR and RT-PCR products revealed the unique 801 bp IκBαM cDNA in AdIκBαM-transfected, but not in AdLacZ-transfected or untransfected, mature DC, indicating that the IκBαM gene is integrated into the genome and transcribed in infected mature DC (Figure 2A). As determined by Western blot analysis, the tagged transgene product can be visualized as being slightly larger than the native IκBα. A dose- and time-dependent increase in IκBαM expression can be seen in AdIκBαM-transfected mature DC, which peaked at an MOI of 100 pfu/cell and at 48 h (Figure 2B).

Inhibition by AdIκBαM of TNF-α-induced NF-κB activation in mature DC The function of AdIκBαM in blocking NF-κB binding was assessed by EMSA. In contrast to a basal NF-κB activity in the control (untransfected and nonstimulated) DC, the TNF-α-stimulated DC exhibited a dramatically promoted NF-κB activity ($P<0.01$). Gene transfer of IκBαM, but not LacZ, at an MOI of 100 pfu/cell and 48 h

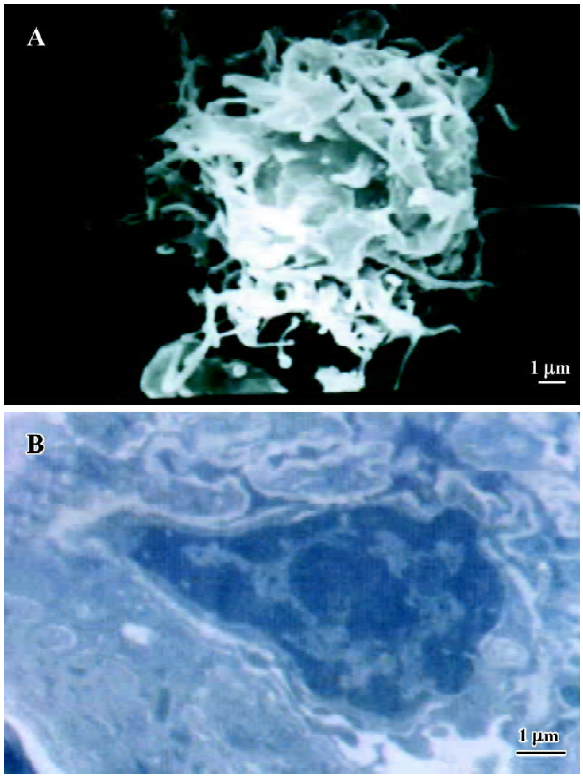

Figure 1. Electron microscopy analysis of morphology of the mature DC. (A) The monocyte-derived mature DC acquired the characteristic dendrites and veiled projections (SEM, ×7000). (B) In addition to ample surface dendrites, the mature DC displayed plentiful cytosolic mitochondria and rough endoplasmic reticula, but few lysosomes and Golgi complexes (TEM, ×12 000).

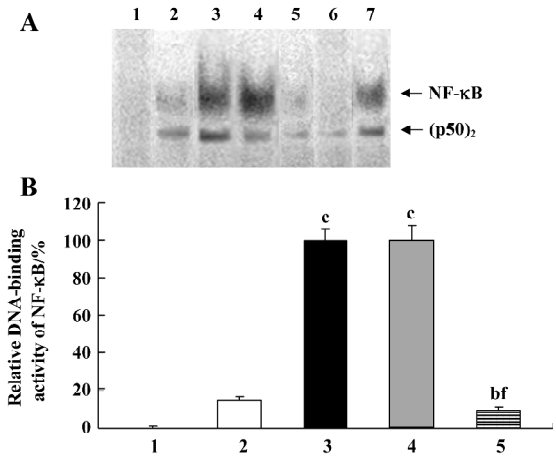

Figure 3. Inhibition by AdIκBαM of TNF-α-induced NF-κB activation in mature DC. (A) EMSA. Lane 1: no nuclear extracts; lane 2: control; lane 3: mature DC stimulated with 10 ng/mL TNF-α for 30 min; lanes 4 and 5: pretreated with 50 pfu/cell of AdLacZ and AdIκBαM for 48 h, respectively, followed by stimulation with 10 ng/mL TNF-α for 30 min. Lanes 6 and 7 (competition assay): addition of a 100-fold excess of unlabeled NF-κB and mutant NF-κB oligonucleotide probes, respectively. Note that the nuclear extracts of lanes 4–7 were derived from those of lane 3. The free DNA probe is not shown. (B) Relative DNA-binding activity of NF-κB in EMSA. n=3. Data are mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* the control group; $^{f}P<0.01$ *vs* the TNF-α-stimulated group.

sharply inhibited the NF-κB activity compared with the TNF-α-stimulated DC ($P<0.01$), suggesting that AdIκBαM inhibits TNF-α-induced NF-κB activation in mature DC (Figure 3A, 3B). In competition assays, addition of 100-fold excess of unlabeled NF-κB, but not mutant NF-κB, oligonucleotide affected NF-κB binding, confirming the specificity of NF-κB binding (Figure 3A).

Induction by AdIκBαM of apoptosis in mature DC Flow cytometry revealed low levels of normal apoptosis in mature DC on d 7, with a higher level of apoptosis 48 h later ($P<0.01$). Optimal gene transfer of IκBαM, but not LacZ, significantly augmented the apoptosis of mature DC ($P<0.01$), demonstrating that AdIκBαM facilitates apoptosis in mature DC (Figure 4).

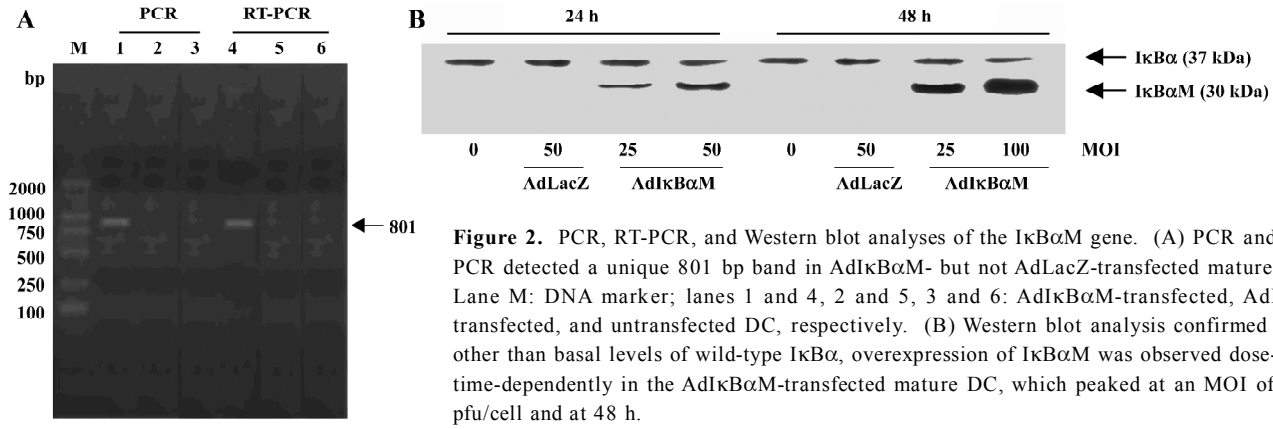

Figure 2. PCR, RT-PCR, and Western blot analyses of the IκBαM gene. (A) PCR and RT-PCR detected a unique 801 bp band in AdIκBαM- but not AdLacZ-transfected mature DC. Lane M: DNA marker; lanes 1 and 4, 2 and 5, 3 and 6: AdIκBαM-transfected, AdLaZ-transfected, and untransfected DC, respectively. (B) Western blot analysis confirmed that, other than basal levels of wild-type IκBα, overexpression of IκBαM was observed dose- and time-dependently in the AdIκBαM-transfected mature DC, which peaked at an MOI of 100 pfu/cell and at 48 h.

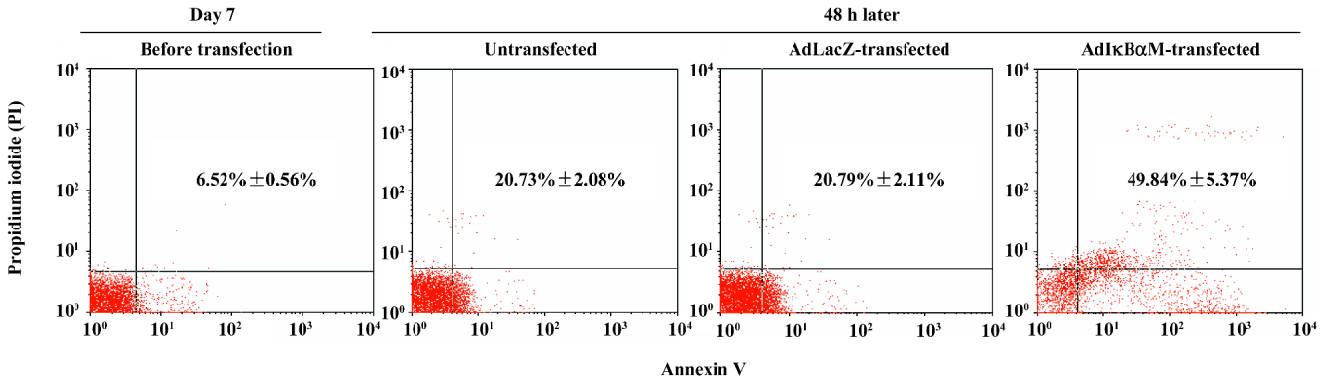

Figure 4. Induction by AdIκBαM of apoptosis in mature DC (dotplot, flow cytometry). LPS-stimulated mature DC exhibited normal low levels of apoptosis on d 7, together with an augmentation of apoptosis 48 h later. Overexpression of IκBαM, but not LacZ, sharply promoted the apoptosis of mature DC. n=4. Data are mean±SD. One of 4 independent experiments is shown.

Effects of AdIκBαM on phenotype and function of mature DC Flow cytometry revealed low levels of CD14, but high levels of HLA-DR, CD83, CD80, and CD86 in mature DC. Except for similar levels of CD14 and HLA-DR (P>0.05), optimal gene transfer of IκBαM, but not LacZ, greatly downregulated the levels of CD83, CD80, and CD86 in mature DC, among which CD80 had a smaller decrease than did CD86 (all P<0.01). Notably, the mature DC overexpressing IκBαM still secreted high levels of CD83, which is a hallmark of maturation. These results imply that downregulation of the B7 surface molecules by AdIκBαM in mature DC may contribute to the immune shift of T cells (Figure 5, Table 1). ELISA confirmed that gene transfer of IκBαM, but not LacZ, resulted in a lower secretion levels of IL-12 (p70) in mature DC (P<0.01, Figure 6). The mixed leukocyte reaction revealed, in parallel with the ratio of DC:T cells, a similar potency for stimulating the proliferation of allogeneic T cells between AdLacZ-transfected and untransfected mature DC. In contrast, the AdIκBαM-transfected mature DC embodied a strikingly decreased mixed leukocyte reaction in comparison with the AdLacZ-transfected mature DC (P<0.05 or 0.01; Figure 7).

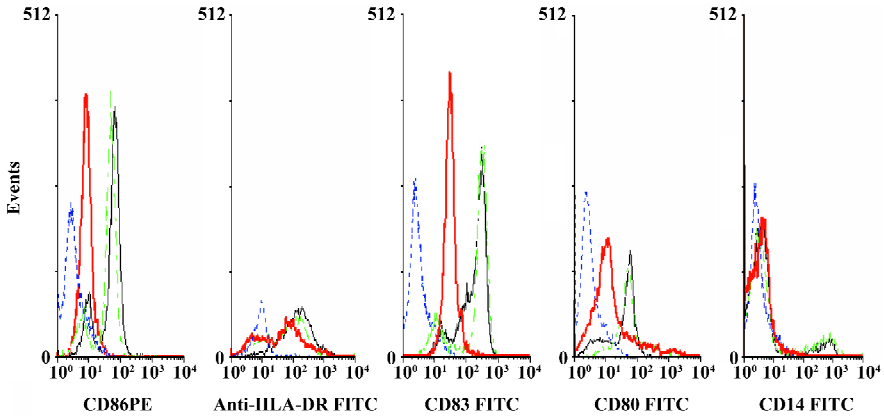

Figure 5. Effect of AdIκBαM on the phenotype of mature DC (histogram, flow cytometry). Both control and AdLacZ-transfected mature DC expressed low levels of CD14, and high levels of HLA-DR, CD83, CD80, and CD86. Overexpression of IκBαM, but not LacZ, greatly downregulated the levels of CD83, CD80, and CD86, except for CD14 and HLA-DR. Blue: isotype-matched DC; red: AdIκBαM-transfected DC; green: AdLacZ-transfected DC; black: untransfected control DC. One of 6 independent experiments is shown.

Table 1. Effect of AdIκBαM on surface markers in mature DC (%). n=6. Data are mean±SD. ^{b}P<0.05, ^{c}P<0.01 *vs* the untransfected group; ^{f}P<0.01 *vs* the AdLacZ-transfected group.

Groups	CD14	HLA-DR	CD83	CD80	CD86
Untransfected	6.8±1.2	91.2±4.3	86.7±5.9	90.8±3.5	93.7±4.0
AdLacZ-transfected	7.1±1.0	92.5±6.1	85.8±6.3	92.4±4.4	95.2±3.5
AdIκBαM-transfected	7.0±1.3	94.3±5.7	78.5±5.4^{b}	80.2±4.8^{cf}	61.9±4.6^{cf}

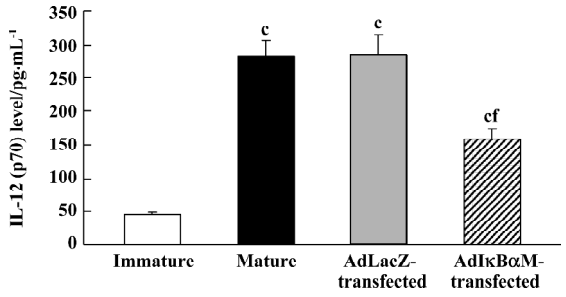

Figure 6. Suppression by AdIκBαM of IL-12 (p70) secretion levels in mature DC (ELISA). Immature DC secreted low levels of IL-12 (p70), but the LPS-stimulated mature DC had higher levels of IL-12 (p70). Overexpression of IκBαM, but not LaZ, significantly suppressed the secretion levels of IL-12 (p70). n=3. Data are mean±SD. $^{c}P<0.01$ *vs* the immature DC; $^{f}P<0.01$ *vs* the mature DC.

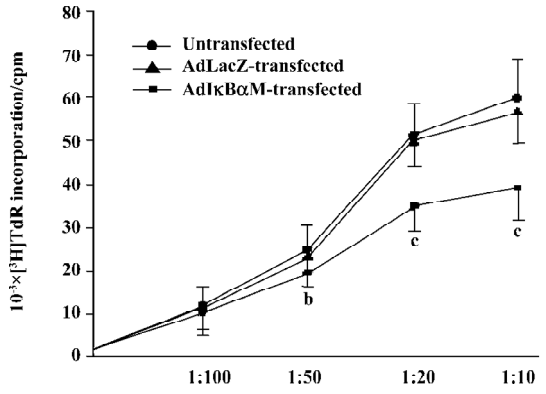

Figure 7. Prohibition by AdIκBαM of DC-stimulated proliferation of T cells (mixed leukocyte reaction). Both AdLacZ-transfected and untransfected mature DC exerted similar potency for stimulating the proliferation of T cells in proportion to the ratio of DC:T cells. However, the MLR of AdIκBαM-transfected DC was significantly ameliorated relative to that of the AdLacZ-transfected DC. n=3. Data are mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* the untransfected or AdLacZ-transfected mature DC.

Discussion

T-cell stimulation and Th1/Th2-cell polarization require 3 DC-derived signals: signal 1 (recognizing signal), which is mediated through T-cell receptor (TCR) triggering by MHC class II-associated peptides processed from pathogens; signal 2 (costimulatory signal), which is mainly mediated by triggering of T cell CD28 by CD80 (B7-1) and CD86 (B7-2); and signal 3 (polarizing signal), which is mediated by various soluble or membrane-bound factors[13–15].

NF-κB plays a crucial role in antiapoptosis by regulation of the transcription of cytokine genes, induction of the antiapoptotic genes, and promotion of the TNF receptor-associated factors and the inhibitor-of-apoptosis proteins to block the activation of caspase-8[16,17]. IκBα, a 37 kDa inhibitory protein, is arranged in 3 domains: the N-terminal domain-containing specific phosphorylation sites, the internal domain of 5 tandem ankyrin repeat sequences, and the C-terminal domain containing Pro-Glu-Ser-Thr polypeptides for regulation of NF-κB activity, binding of NF-κB, and rapid protein turnover, respectively[18]. IκBα retains the p65-containing NF-κB complex in the cytoplasm by masking its nuclear localization sequence (NLS). Upon appropriate stimulation, for example by IL-1β, TNF-α, or LPS, IκBα undergoes phosphorylation on two serine residues, Ser 32 and Ser 36, which renders IκBα susceptible to proteolytic degradation via the ubiquitin-proteasome pathway, resulting in release and nuclear translocation of NF-κB. Consequently, the active NF-κB, singlely or in combination with other nuclear factors, initiates transcription of target genes associated with immune and inflammatory responses. As for the inflammatory cascade, NF-κB repression in airways through suppression of IκBα degradation or augmentation of IκBα synthesis can downregulate transcription of an array of the NF-κB-dependent genes, which is more effective than blockade of single downstream inflammatory or immune genes[19].

In the present study, monocytes cocultured with GM-CSF and IL-4 differentiated into mature DC upon stimulation with maturation inducer LPS. The IκBαM gene was successfully delivered into the monocyte-derived mature DC, and was expressed time- and dose-dependently. Importantly, NF-κB-blocked mature DC by AdIκBαM obviously inhibited the proliferation of T cells, which is in agreement with the report that selective inhibition of NF-κB in DC by the NF-κB essential modulator-binding domain peptide blocks maturation and prevents T-cell proliferation, accompanied by less Th1 and Th2 polarization[20]. Furthermore, TNF-α-induced NF-κB activation of mature DC was significantly attenuated by AdIκBαM, and the NF-κB-blocked mature DC were more susceptible to apoptosis than those transfected with AdLacZ. Nevertheless, blockade of NF-κB in mature DC, other than little change in HLA-DR related to antigen-presentation, caused striking downregulation of the B7 costimulatory molecules. It is deduced, therefore, that AdIκBαM might, in part, facilitate the antigen-specific immune tolerance of T cells via induction of DC apoptosis and downregulation of B7 molecules.

Asthma is, *per se*, a multigenetically susceptible clinical syndrome, in which sensitized individuals develop an aberrant Th2-dominated immunity for the underlying basis of eosinophilic airway inflammation[10,14]. B7-1 and B7-2 differentiate the Th0 cells into a Th1 subset for cellular immunity

and a Th2 subset for humoral immunity, respectively[1,13,15]. Interestingly, downregulation of B7-2 was more effective than that of B7-1 in the mature DC overexpressing IκBαM, indicating that blockade of NF-κB in DC may, to some extent, contribute to rectifying the Th1/Th2 imbalance in asthmatics. It is noteworthy that Th2 development could be the default pathway induced by DC when the production of IL-12 is quite low[2]. As the major effective treatment for asthma, corticosteroids, like all pharmacologic agents that have been shown to inhibit NF-κB, have numerous other effects that could limit their therapeutic usefulness[21]. Currently, there is much interest in identifying more specific and effective NF-κB inhibitors, among which the IκBαM is a novel NF-κB inhibitor. Although blocking the NF-κB pathway is unlikely to be a clinically beneficial approach due to the broad range of genes involved, it is of importance to understand the role of NF-κB during specific immune responses for identifying relevant molecules as potential therapeutic targets[22].

In summary, our findings suggest that the AdIκBαM is applicable to both effective gene transfer of IκBαM and specific blockade of NF-κB in monocyte-derived mature DC. Moreover, the ability of mature DC overexpressing IκBαM to induce DC apoptosis and downregulate B7 molecules highlights an important mechanism for T-cell immune suppression. The successful downregulation of T-cell responses following inhibition of NF-κB in mature DC offers a potential strategy for future immunotherapy of asthma[23,24]. Antigen-specific T-cell immune tolerance could be induced by selective blockade of NF-κB with AdIκBαM in antigen-primed mature DC (ie by pollen, ragweed, house dust mite, etc). Alternatively, the DC-mediated Th1-dominated response is promising for amelioration of the undue Th2 by AdIκBαM in combination with Th1-inducing agents, such as CpG oligodeoxynucleotide, purified protein derivative and bacilli Calmette-Guerin[25,26].

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