Roles of Bcl-2 and caspases in anti-CD30 monoclonal antibody-induced primary human eosinophil apoptosis

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ABSTRACT

Roles of Bcl-2 and caspases in anti-CD30 monoclonal antibody-induced primary human eosinophil apoptosis

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Background: *In vitro* studies have suggested that activation of cell surface CD30 by immobilized anti-CD30 monoclonal antibodies (mAb) induces strong apoptosis in human eosinophils. This anti-CD30 mAb-induced eosinophil apoptosis was inhibited by the addition of inhibitors of p38, ERK1/2 mitogen-activated protein kinases (MAPKs), and phosphatidylinositol 3-kinase (PI3K). However, there is little data investigating the role of Bcl-2 and caspases in eosinophil apoptosis induced by anti-CD30 mAb.

Objectives: We sought to determine whether anti-CD30 mAb induces human eosinophil apoptosis via Bcl-2 and caspases pathways.

Methods: Peripheral blood was drawn from 19 healthy volunteers. Purified human eosinophils were suspended in RPMI 1640 media supplemented with 10% FBS. The CD30 expression on eosinophils was measured at various time points. Eosinophils were then cultured in plates precoated with anti-CD30 mAb (clone Ber-H8), isotype control immunoglobulin G_1, interleukin (IL)-5, or dexamethasone. Western blot analysis was performed to determine the expression of Bcl-2, procaspase-8, -9, and -3, and caspase-8, -9, and -3 after cross-linking of CD30. Human eosinophils were also cultured in plates precoated with anti-CD30 mAb (clone Ber-H8) in the presence or absence of caspase-9 or -3 inhibitors. Eosinophil apoptosis was assessed using flow cytometry.

Results: The addition of anti-CD30 mAb significantly increased eosinophil apoptosis *in vitro* compared with controls. In western blot analysis, the addition of anti-CD30 mAb significantly decreased the expression of Bcl-2 and procaspase-9 and -3 and increased the expression of caspase-9 and -3. The addition of caspase-9 or -3 inhibitors at reasonable concentrations decreased anti-CD30 mAb-induced human eosinophil apoptosis *in vitro*. Procaspase-8 or caspases-8 expression was not changed in response to various stimuli.

Conclusions: Anti-CD30 mAb-induced human eosinophil apoptosis is likely to be mediated through Bcl-2 and caspase-9 and -3. Future studies are warranted to

delineate downstream signaling molecules in the Bcl-2 family and caspases in human eosinophil apoptosis.

Key words: CD30, eosinophils, apoptosis, Bcl-2, caspase, allergy

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I. INTRODUCTION

Eosinophils are resident in various organs such as the respiratory tract, the gastrointestinal tract, mammary glands, and bone marrow. They may play an important role in these organs and in maintaining immune homeostasis of these organs.[1] In T-helper 2 (Th_2)-type immune response, eosinophils are recruited into inflammation sites, where they produce various kinds of cytokines and chemokines and release toxic granule proteins in response to diverse physiological and artificial stimuli.[1] These molecules may regulate inflammatory immune responses, cause tissue damage, and promote tissue repair.[2] Eosinophils can also present antigens to naive and memory T cells and trigger and/or intensify antigen-specific immune responses.[1]

Given that eosinophils play essential roles in immune responses, such as allergic diseases, as both effector and modulatory cells, many studies have been conducted to reduce allergic inflammation by selectively inducing apoptosis of eosinophils.[3-7] The focus of the previous studies was directed toward the receptors found on the surface of human eosinophils in an attempt to induce eosinophil apoptosis by cross-linking of the

surface molecules.[3-9]Among them, CD30, a member of the tumor necrosis factor receptor (TNFR) family, has been found to exist on the eosinophil surface.[3,4,7,10] Although there have been a few studies demonstrating that cross-linking of CD30 on the surface of human eosinophils can induce apoptosis through a tightly regulated intracellular signaling pathway, limited studies have been carried out to determine the actual role of CD30 molecules on human eosinophils.[3,4,7,10]

Neutrophils, mast cells, T cells, and dendritic cells are important effector cells in allergic diseases. Many proteins are known to play a role in regulating human cell apoptosis. Studies have shown that Bcl-2[11-15] and caspases[16-19] play a crucial role in apoptosis in various types of human hematopoietic cells. However, there are few studies in the literature clarifying the possible role of Bcl-2 and caspases in human eosinophil apoptosis after cross-linking of CD30.

Therefore, in the present *in vitro* study, we sought to determine whether Bcl-2 and caspase-8, -9 and -3 are involved in the signal transduction pathways in human eosinophil apoptosis through cross-linking of CD30.

II. MATERIALS AND METHODS

1. Human eosinophil preparation

Eosinophils were purified from the peripheral blood of healthy subjects (n = 19) by Percoll-sodium diatrizoate density gradient (Amershan Pharmacia Biotech, Uppsala, Sweden) sedimentation and negative selection with anti-CD16 antibody-coated

immunomagnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany) using the FACS Flow Cytometry (Becton Dickinson Co., Mountain View, CA, USA), as previously described.[20] Both the purity and viability of the eosinophils were confirmed to exceed 98% based on light microscopic examination of cytocentrifuged preparations by Randolph stain and trypan blue (Sigma-Aldrich) dye exclusion, respectively. Purified eosinophils were suspended at a cell density of 1 X 10^6/mL in Roswell Park Memorial Institute (RPMI) 1640 media (Atlanta Biologicals) supplemented with 10% FBS (Atlanta Biologicals) and 100 μg/mL streptomycin. Written informed consent was obtained from all subjects, and the study was approved by the institutional review board. All experiments were performed in triplicate.

2. Human eosinophil culture

Purified eosinophils were suspended at a cell density of 1 X 10^6/mL in RPMI 1640 media (Atlanta Biologicals) supplemented with 1% or 10% FBS (Atlanta Biologicals) and 100 μg/mL streptomycin. Anti-CD30 agonistic monoclonal antibodies (mAb), Ber-H8 (BD Pharmingen, San Diego, CA, USA), and control IgG_1 (Santa Biotechnology Santa Cruz, CA, USA) were used to investigate the effect of CD30 activation on eosinophil apoptosis. Ber-H8 and IgG_1 were suspended in PBS at a concentration of 20 μg/mL and 500 μL/well were transferred to flat bottom 24 well plates (Costar, Cambridge, MA, USA). After the plates were precoated and immobilized with the mAb at 4℃ for 24 hours, wells were washed three times with PBS containing 1% BSA and blocked with 1 mL of 1% BSA at

4℃ overnight and then equal numbers of cells were supplemented. Each experimental group was incubated in the RPMI 1640 supplemented with 10% heat-inactivated FBS at different wells in a 37℃ humidified atmosphere with 5% CO_2 for 0, 4, 24, 66, or 90 hours. The groups were classified into media only, 10 ng/mL IL-5, 10 μM/mL dexamethasone (Sigma Chemical Co. St. Louis, USA), 20 μg/mL Ber-H8, 20 μg/mL IgG_1, 20 μg/mL Ber-H8 + 10 ng/mL IL-5, and 20 μg/mL IgG_1 + 10 ng/mL IL-5. Additionally, some purified eosinophils were also cultured in the presence of caspase-9 inhibitors (BD Pharmingen, San Diego, CA, USA) or caspase-3 inhibitors (BD Pharmingen, San Diego, CA, USA) for 24 and 48 hours and the cells were collected and apoptosis was measured by flow cytometry.

3. Expression of CD30 on the surface of human eosinophils

The expression of CD30 on eosinophils was examined using indirect immunofluorescence and flow cytometry, as previously described.[4] Freshly isolated eosinophils are labeled (30 min, 4℃) in PBS containing 1% BSA (PBS-BSA; Sigma-Aldrich) and 4 mg/mL human IgG (Sigma-Aldrich) with a saturating concentration of anti-CD30 mAb or an equivalent concentration of irrelevant isotype-matched control mAb. Cells were washed and then incubated (30 min, 4℃ in PBS-BSA) with appropriate dilutions of FITC-conjugated $F(ab')_2$ goat anti-mouse IgG Ab (BD sciences, Mountain View, CA, USA). After fixation in 1% paraformaldehyde in PBS, fluorescence analysis was conducted in the FACS Flow Cytometry. Fluorescence intensity was determined on

20,000 cells from each sample using logarithmic amplification, which was converted to the linear equivalent by the Lysis II software (FACScan, Becton Dickinson Co., Mountain View, CA, USA).

4. Assessment of human eosinophil apoptosis

The Annexin V-FITC/propidium iodide (PI) Apoptosis Detection Kit (BD Pharmingen, San Diego, CA, USA) was used according to the manufacturer's instructions.[21] Briefly, cultured eosinophils were harvested at various time points by gentle pipetting and washed once with PBS. The harvested eosinophils were then suspended in a Ca^{2+}-containing buffer and reacted with FITC-conjugated Annexin V for 15 min at room temperature. One minute before FACS Flow Cytometry analysis, the eosinophils were also stained with 10 μg/mL PI. The FITC-stained cells (Annexin V-positive) and PI-stained cells were assessed by the FACS Flow Cytometry, as previously described.[4]

5. Assessment of Bcl-2, procaspase-8, -9, and -3, and caspase-8, -9, and -3 expressions in human eosinophils by western blotting

Cultured eosinophils were collected at 24 hours and protein was extracted using RIPA Lysis and Extraction Buffer. After centrifugation at 4℃, 13,000 rpm for 20 min, protein concentration was measured by Bradford method and diluted with 5X SDS sample buffer, and then boiled the diluted protein at 100°C for 5 min. Protein concentrate of 1 μg/μLwas

inoculated to 12.5% polyacrylamide gel, transferred to polyvinylidene fluoride membrane and separated for one hour by 100 mV. The membrane was fixed at room temperature for one hour in the solution mixed with Tween-20 Tris-buffered saline dissolved in nonfat dry milk. The primary antibody (Bcl-2, procaspase-8, -9, and -3, and caspase-8, -9, and -3) was combined to the membrane and was reacted at 4℃ overnight. The membrane was washed with TBS/Tween-20 four times strongly at room temperature and reacted with secondary antibody at room temperature for 2 hours. We assessed Bcl-2, procaspase-8, -9, and -3, and caspase-8, -9, and -3 proteins using western blotting luminal reagent kit and exposed them to the Kodak BioMax Light Film and developed it by Kodak GBX Developer and Replenisher.

6. Statistical analysis

All data were presented as mean ± SEM unless otherwise indicated. Differences between groups were analyzed using the Mann-Whitney *U* test or the Independent *t*-test. All analyses were conducted by using IBM SPSS Statistics for Windows, Ver. 21.0 (IBM Corp., Armonk, NY, USA). Allstatistical tests were two-sided, and $P<0.05$ represented statistical significance.

III. RESULTS

1. Expression of CD30 on primary human eosinophils cultured in different conditions

Indirect immunofluorescence and flow cytometry were used to examine CD30 expression on eosinophils from nineteen different donors. Freshly isolated peripheral blood eosinophils were found to express low, but consistently detectable, amounts of CD30. The percentages of eosinophils expressing CD30 in RPMI 1640 media with 1% FBS, 10% FBS, and IL-5 + 10% FBS at 24 hr of culture assessed by flow cytometry were 5.6 ± 1.1%, 3.5 ± 1.7%, and 3.5 ± 1.5%, respectively. The human eosinophils showed lower CD30 expression when cultured with 10% FBS as compared with 1% FBS at 24 hours. Addition of IL-5 to eosinophils cultured in RPMI 1640 media with 10% FBS showed no difference when compared with eosinophils cultured in RPMI 1640 media with 10% FBS only.

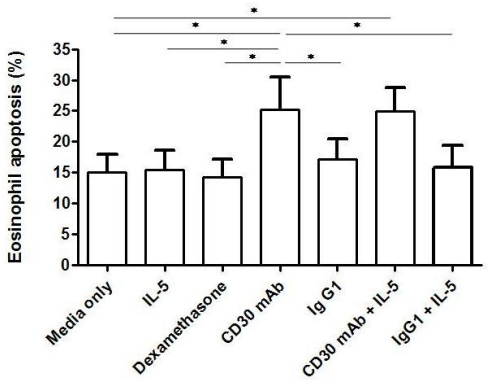
2. Expression of CD30 on human eosinophils at various time points

Indirect immunofluorescence and flow cytometry were used to examine CD30 expression on eosinophils incubated in RPMI 1640 media supplemented with 10% FBS from nineteen different donors at various time points. The percentages of eosinophils expressing CD30 at 0, 24, 66, and 90 hr of culture assessed by flow cytometry were 1.3 ± 0.4%, 2.4 ± 0.4%, 3.5 ± 1.7%, 8.3 ± 0.7%, and 17.3 ± 0.8%, respectively ($P < 0.05$). The CD30 expression on human eosinophils increased in a time-dependent manner.

3. Effects of immobilized anti-CD30 mAb on eosinophil apoptosis

Eosinophils were cultured in 24-well plates with media only, IL-5 10 ng/mL, dexamethasone 10 μM/mL, Ber-H8 (anti-CD30 mAb) 20 μg/mL, IgG_1 20 μg/mL, Ber-H8 20 μg/mL + IL-5 10 ng/mL, and IgG_1 20 μg/mL + IL-5 10 ng/mL, respectively. The eosinophil apoptosis rate (annexin V-positive eosinophils) was determined at 24 and 48 hr. As shown in Figure 1A, eosinophils cultured in the presence of immobilized anti-CD30 mAb, Ber-H8, resulted in increased apoptosis (annexin V-positive eosinophils) that was significant at 24 hr of culture (25.2 ± 5.3%), when compared with eosinophils cultured with media only, IL-5, dexamethasone, IgG_1, or IgG_1 mAb + IL-5(Figure 1A). At 48 hr of culture, culture of eosinophils in the presence of immobilized anti-CD30 mAb, Ber-H8, resulted in increased apoptosis (annexin V-positive eosinophils) when compared with eosinophils cultured with media only or IL-5 (Figure 1B).

A)



B)

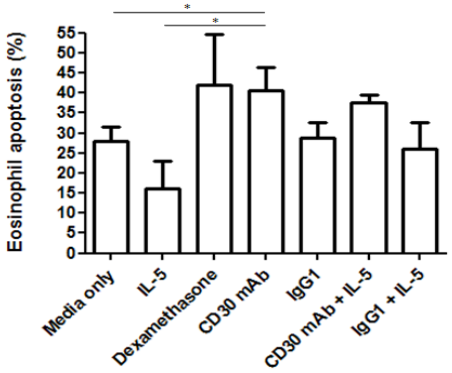


Figure 1. Effects of immobilized anti-CD30 mAb on eosinophil apoptosis measured at 24 hr (A) and 48 hr (B). $^{*}P<0.05$, analyzed by a Mann-Whitney *U* test.

4. Expression of procaspase-8 and caspase-8 assessed by western blot analysis of human eosinophil extracts after various stimuli

Eosinophils were cultured in IgG_1-coated plates, IL-5, or anti-CD30 mAb (Ber-H8)-coated plates and the procaspase-8 and caspase-8 expression was assessed at 24 hr by western blotting (Figure 2). Pretreatment of eosinophils with IgG_1 20 μg/mL, IL-5 10 ng/mL, or anti-CD30 mAb (Ber-H8) 20 μg/mL for 24 hr did not change the procaspase-8 or caspases-8 expression levels (Figure 2).

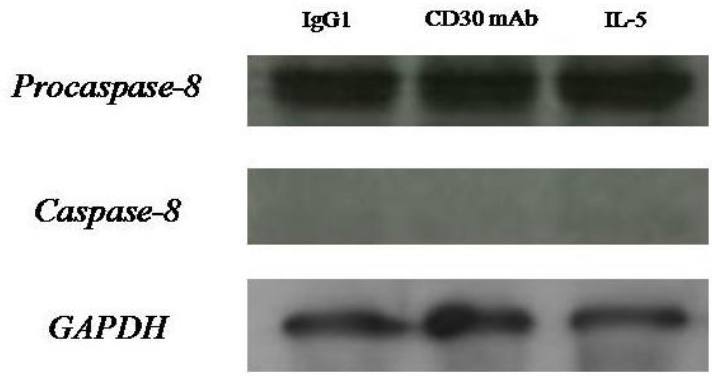


Figure 2. Western blot analysis of procaspase-8 and caspase-8 expression in human eosinophil extracts at 24 hr after various stimuli.

5. Expression of Bcl-2 assessed by western blot analysis of human eosinophil extracts after various stimuli

Eosinophils were cultured in IgG_1-coated plates, anti-CD30 mAb (Ber-H8)-coated plates + IL-5, IL-5, or anti-CD30 mAb (Ber-H8)-coated plates and the Bcl-2 expression was

assessed at 24 hr by western blotting (Figure 3). Pretreatment of eosinophils with 10 ng/mL of IL-5 for 24 hr significantly increased the Bcl-2 expression, whereas anti-CD30 mAb (Ber-H8) decreased the Bcl-2 expression (Figure 3).

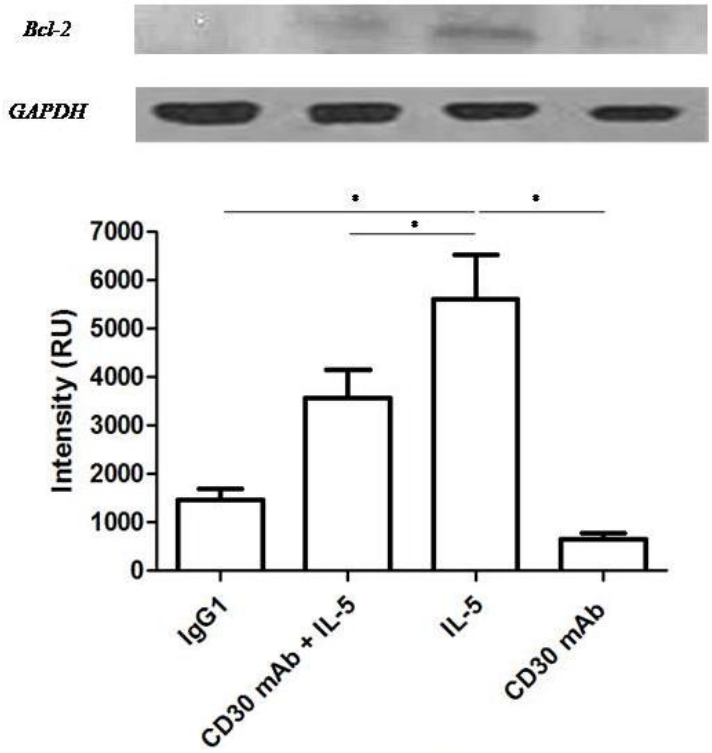


Figure 3. Western blot analysis of Bcl-2 expression in human eosinophil extracts at 24 hr after various stimuli. $^{*}P<0.05$, analyzed by a Mann-Whitney *U* test.

6. Expression of procaspase-9 and procaspase-3 assessed by western blot analysis of human eosinophil extracts after various stimuli

Eosinophils were cultured in IgG_1-coated plates, IL-5, or anti-CD30 mAb (Ber-H8)-coated plates and the procaspase-9 expression was assessed at 24 hr by western blotting. Pretreatment of eosinophils with 10 ng/mL of IL-5 for 24 hr significantly increased the

procaspase-9 expression, whereas anti-CD30 mAb (Ber-H8) significantly decreased the procaspase-9 expression (Figure 4). Pretreatment of eosinophils with 10 ng/mL of IL-5 for 24 hr significantly increased the procaspase-3 expression, whereas anti-CD30 mAb (Ber-H8) significantly decreased the procaspase-3 expression (Figure 5).

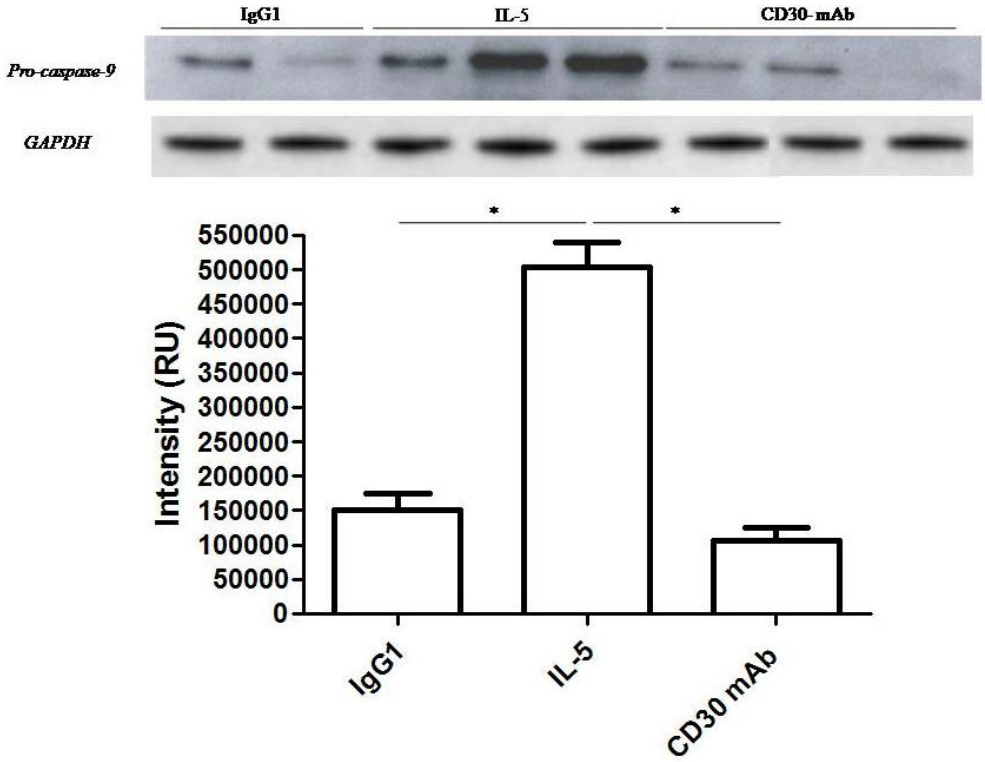


Figure 4. Western blot analysis of procaspase-9 expression in human eosinophil extracts at 24 hr after various stimuli. $^{*}P<0.05$, analyzed by a Mann-Whitney *U* test.

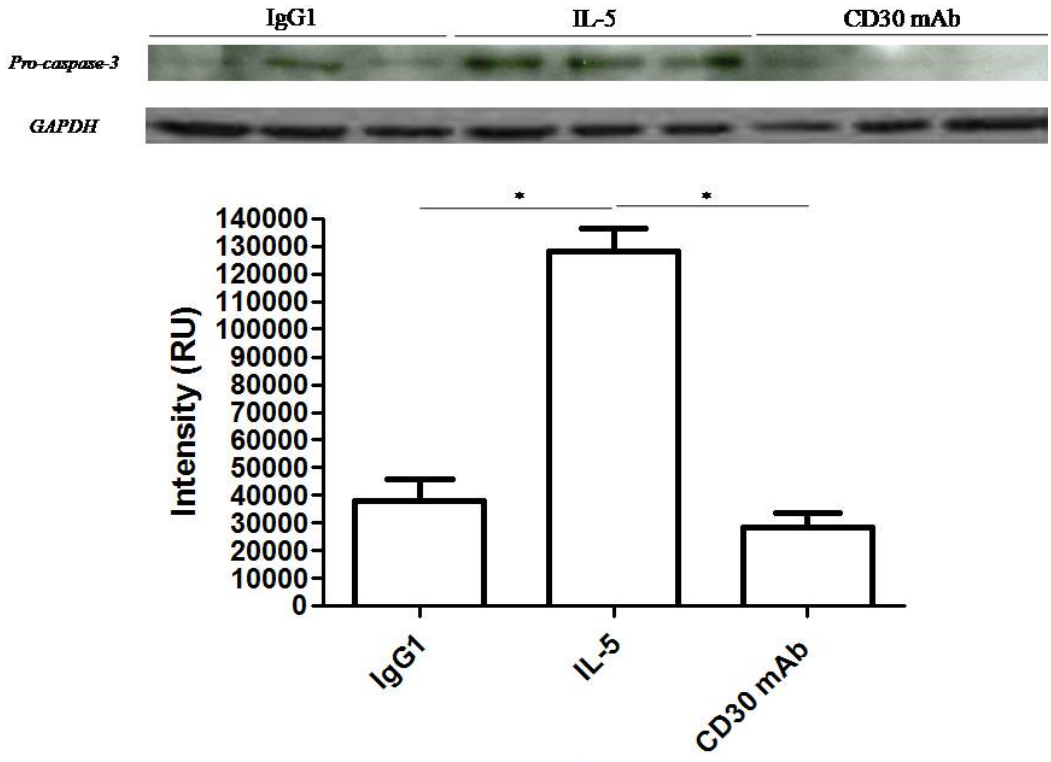


Figure 5. Western blot analysis of procaspase-3 expression in human eosinophil extracts at 24 hr after various stimuli. $^{*}P<0.05$, analyzed by a Mann-Whitney *U* test.

7. Effects of caspase-9 and caspase-3 inhibitors on the expression of caspase-9 and caspase-3 assessed by western blot analysis of human eosinophil extracts after various stimuli

Eosinophils were cultured in IgG_1-coated plates, IL-5, anti-CD30 mAb (Ber-H8)-coated plates + caspase-9 or caspases-3 inhibitors, or anti-CD30 mAb (Ber-H8)-coated plates and the caspase-9 and caspase-3 expression was assessed at 24 hr by western blotting. Pretreatment of eosinophils with anti-CD30 mAb (Ber-H8) significantly increased the

caspase-9 expression, whereas anti-CD30 mAb (Ber-H8) + caspase-9 inhibitor decreased the caspase-9 expression (Figure 6). Pretreatment of eosinophils with anti-CD30 mAb (Ber-H8) significantly increased the caspase-3 expression, whereas anti-CD30 mAb (Ber-H8) + caspase-3 inhibitor decreased the caspase-3 expression (Figure 7).

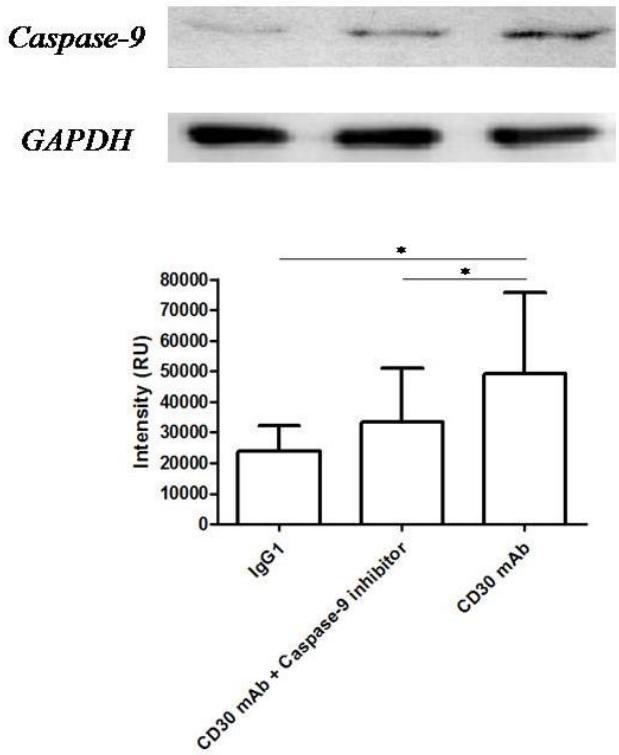


Figure 6. Western blot analysis of caspase-9 expression in human eosinophil extracts at 24 hr after treatment of caspase-9 inhibitor. $^{*}P<0.05$, analyzed by a Mann-Whitney *U* test.

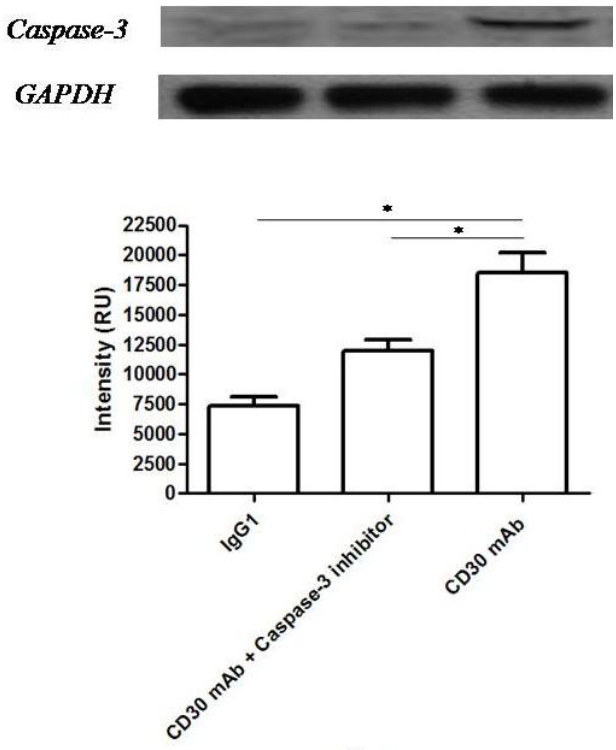


Figure 7. Western blot analysis of caspase-3 expression in human eosinophil extracts at 24 hr after treatment of caspase-3 inhibitor. $^{*}P<0.05$, analyzed by a Mann-Whitney *U* test.

8. Effects of caspase-9 and caspase-3 inhibitors on immobilized anti-CD30 mAb-induced eosinophil apoptosis

Eosinophils were cultured in media only, anti-CD30 mAb (Ber-H8)-coated plates, anti-CD30 mAb (Ber-H8)-coated plates + caspase-3 inhibitor, anti-CD30 mAb (Ber-H8)-coated plates + caspase-9 inhibitor, or IgG_1 for 24 and 48 hr, respectively (Figure 8A and B). The rate of annexin V positivity was higher in the eosinophils cultured in the anti-

CD30 mAb (Ber-H8)-coated plates than in the eosinophils cultured in either media only or irrelevant control IgG_1-coated plates at 24 and 48 hr, respectively.

In order to determine the involvement of caspase-9 in this apoptosis, caspase-9 inhibitors were added in anti-CD30 mAb (Ber-H8)-coated plates. Addition of 20 μM of caspase-9 inhibitors showed a reduction in anti-CD30 mAb (Ber-H8)-induced eosinophil apoptosis at 24 and 48 hr, respectively (Figure 8A and B). In order to determine the involvement of caspase-3 in this apoptosis, caspase-3 inhibitors were added in anti-CD30 mAb (Ber-H8)-coated plates. Addition of 20 μM of caspase-3 inhibitors showed a significant reduction in anti-CD30 mAb (Ber-H8)-induced eosinophil apoptosis at 24 and 48 hr, respectively (Figure 8A and B).

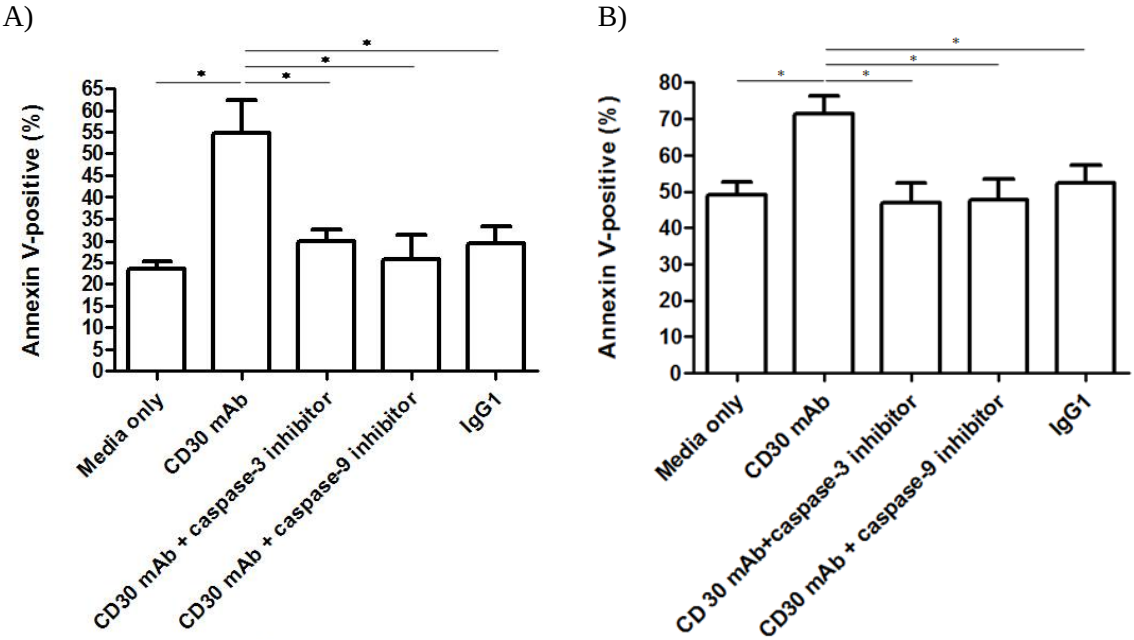


Figure 8. Effects of caspase-3 and caspase-9 inhibitors on immobilized anti-CD30 mAb-induced eosinophil apoptosis at 24 hr (A) and 48 hr (B). $^{*}P<0.05$, analyzed by a Mann-Whitney *U* test.

IV. DISCUSSION

In the present *in vitro* study, we sought to determine whether Bcl-2 and caspase-8, -9 and -3 are involved in the signal transduction pathways in human eosinophil apoptosis through cross-linking of CD30 and found that Bcl-2 and caspase-9 and -3 are critically engaged in the signal transduction pathways in human eosinophil apoptosis through cross-linking of CD30. To the best of our knowledge, this is the first study to determine that Bcl-2 and caspase-9 and -3 may play a pivotal role in human eosinophil apoptosis through cross-linking of CD30.

Our findings that activation of cell surface CD30 by immobilized anti-CD30 mAb induces intensified apoptosis in human eosinophils *in vitro* are in good agreement with previous studies.[3,4,7] This effect was observed only when the anti-CD30 mAb was immobilized, not in soluble form, thereby proposing that cross-linking of cell surface molecule CD30 or a second signal may be imperative for the induction of apoptosis.[4]

We investigated whether anti-CD30 mAb (clone Ber-H8) treatment induces human eosinophil apoptosis. As shown in Figure 1, anti-CD30 mAb (clone Ber-H8) induced strong human eosinophil apoptosis when compared with the other stimulators, which correlates well with previous studies.[3,4,7]An important finding worth mentioning in the present study is that anti-CD30 mAb (clone Ber-H8) induced stronger human eosinophil apoptosis than dexamethasone, a well-known potent eosinophil apoptosis inducer,[22] thereby suggesting that anti-CD30 mAb may well be a promising drug candidate for regulating human eosinophil survival in the treatment of allergic diseases in the future.

We determined whether caspase-8 is involved in the signal transduction pathways in

eosinophil apoptosis after anti-CD30 mAb treatment. Procaspase-8 and caspase-8 expression levels were not changed when stimulated with IgG_1, anti-CD30 mAb, or IL-5, suggesting that caspase-8 pathway is not involved in the anti-CD30 mAb-induced human eosinophil apoptosis. Previous studies have suggested that several surface molecules such as Fas are capable of inducing apoptosis in human eosinophils through cross-linking.[6] Fas receptor cross-linking results in death-inducing signaling complex (DISC) assembly initiating within minutes of cross-linking and localizing initially Fas associated death domain (FADD) and then caspase-8 to the receptor complex.[23] Although we did not analyze the activity of death domain after cross-linking of CD30, it can be inferred that CD30, unlike Fas, lacks an intracellular death domain capable of activating the caspase-8 pathway.

We then examined whether Bcl-2 is engaged in the signal transduction pathways in human eosinophil apoptosis after anti-CD30 mAb treatment. As shown in Figure 3, Bcl-2 protein was highly expressed when stimulated with IL-5, an anti-apoptotic protein, whereas its expression was abolished when stimulated with anti-CD30 mAb, a pro-apoptotic protein. The Bcl-2 protein was moderately expressed when stimulated with both IL-5 and anti-CD30 mAb. A previous study has reported that when monocytes were stimulated with a member of the tumor necrosis factor superfamily, apoptosis was inhibited, the Bcl-2 protein level increased, and the caspase-3 level decreased compared to unstimulated control cells.[24] Another study investigating whether a T cell survives or dies following T cell receptor re-engagement found that caspases-mediated cleavage of anti-apoptotic Bcl-2 or Bcl-xL facilitates activation-induced cell death of T cells, suggesting that cleavage of anti-apoptotic Bcl-2 and Bcl-xL contributes to the decision

between T cell activation and apoptosis following TCR re-engagement.[14] Although we did not measure expression of other proteins that may be involved in the mitochondrial pathway, we speculate that the Bcl-2 protein may be engaged in the signal transduction pathways in human eosinophil apoptosis after anti-CD30 mAb treatment.

Next, we explored whether caspase-9 is engaged in the signal transduction pathways in human eosinophil apoptosis after anti-CD30 mAb treatment. As shown in Figure 4, procaspase-9 was highly expressed when stimulated with IL-5, whereas its expression was decreased when stimulated with anti-CD30 mAb. In addition, caspase-9 was highly expressed when stimulated with anti-CD30 mAb. The caspase-9 protein was moderately expressed when stimulated with both anti-CD30 mAb and caspase-9 inhibitor. These findings indicate that upon stimulation by anti-CD30 mAb, procaspase-9 is converted to caspase-9; thus, it can be inferred that caspase-9 pathway is involved in the signal transduction pathways in human eosinophil apoptosis after anti-CD30 mAb treatment.

We also determined whether caspase-3 is involved in the signal transduction pathways in human eosinophil apoptosis after anti-CD30 mAb treatment. As demonstrated in Figure 5, procaspase-3 was highly expressed when stimulated with IL-5, whereas its expression was abrogated when stimulated with anti-CD30 mAb. Furthermore, caspase-3 was highly expressed when stimulated with anti-CD30 mAb. The caspase-3 protein was slightly expressed when stimulated with both anti-CD30 mAb and caspase-3 inhibitor. These results suggest that when stimulated with anti-CD30 mAb, procaspase-3 is converted to caspase-3; thus, we speculate that caspase-3 pathway is involved in the signal transduction pathways in human eosinophil apoptosis after anti-CD30 mAb treatment.

Finally, we determined whether specific inhibitors of caspase-9 or caspase-3 decrease human eosinophil apoptotic effect of anti-CD30 mAb treatment. As shown in Figure 8, caspase-9 and caspase-3 inhibitors significantly decreased the rate of apoptosis in human eosinophils when treated with anti-CD30 mAb, as compared with anti-CD30 mAb only. These data strongly suggest that caspase-9 and caspase-3 are involved in the signal transduction pathways for induction of human eosinophil apoptosis via CD30.

The present study had some limitations. First, we did not analyze tumor necrosis factor receptor associated factor (TRAF)-interacting motifs (TIMs) in human eosinophils, which are activated to recruit TRAF family members, and activation of multiple signal transduction pathways that are important in eosinophil apoptosis. Second, we did not measure various molecules in the Bcl-2 family, which are known to regulate mitochondrial dysfunction.

V. CONCLUSIONS

We found that Bcl-2 and caspase-9 and -3 are critically involved in anti-CD30 mAb-induced human eosinophil apoptosis *in vitro*. Downstream signaling molecules of Bcl-2 and caspase-9 and -3 activations in human eosinophils remain to be clarified in future studies.

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ABSTRACT (IN KOREAN)

항 CD30 단일클론항체에 의한 인체 유래 호산구세포 자멸사에서 Bcl-2와 caspases의 역할

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배경: 이전 연구들은 호산구세포 표면에서 발현되는 CD30을 항 CD30 단일클론 항체로 활성화시키면 호산구세포자멸사가 유도되는 것을 밝혀냈다. 항 CD30 단일클론 항체에 의한 호산구세포자멸사는 p38, ERK1/2 mitogen-activated protein kinases (MAPKs), phosphatidylinositol 3-kinase (PI3K)에 의해서 억제된다. 하지만 항 CD30 단일클론 항체에 의한 호산구 세포 자멸사 과정에서 Bcl-2와 caspases가 어떤 역할을 하는지에 대해서는 알려진 바가 미미하다.

목적: 이번 연구의 목적은 항 CD30 단일클론 항체에 의한 인체 유래 호산구세포 자멸사 과정에서 Bcl-2와 caspases의 역할을 규명하는 것이다.

재료 및 방법: 건강한 성인 19명의 말초혈액에서 호산구를 분리하였고 10% FBS를 포함한 RPMI 1640 미디어에서 배양하였다. 다양한 시간에 호산구 세포표면에서 CD30 표현 정도를 분석하였다. 항 CD30 단일클론항체(Ber-H8), 동형의 immunoglobulin G_1, Interleukin (IL)-5,

덱사메사손을 각각 투여하여 호산구를 배양하였다. 항 CD30 단일클론항체를 투여한 후 Western blot을 이용하여 Bcl-2, procaspase-8, -9, -3, caspase-8, -9, -3 단백질 발현 정도를 분석하였다. 항 CD30 단일클론항체에 의한 호산구자멸사 과정에서 caspases의 역할을 규명하기 위해서 caspase-9 억제제, caspase-3 억제제를 첨가한 후 배양하여 자멸사 율을 분석하였다. 호산구자멸사 율은 flow cytometry를 이용해서 측정하였다.

결과: 항 CD30 단일클론항체를 호산구에 처리하였을 때 호산구자멸사 율이 증가하였다. Western blot 분석에서 항 CD30 단일클론항체를 처리하였을 때 Bcl-2, procaspase-9, procaspase-3 발현이 감소하였고, caspase-9, caspase-3 발현이 증가하였다. 항 CD30 단일클론항체를 투여한 후 caspase-9 억제제나 caspase-3 억제제를 투여하였을 때 호산구자멸사 율이 감소하였다. Procaspase-8과 caspases-8의 발현 정도는 다양한 자극 물질에 대해서 유의한 차이를 나타내지 않았다.

결론: 항 CD30 단일클론항체에 의한 인체 유래 호산구 세포 자멸사는 Bcl-2, caspase-9, caspase-3에 의해서 발생할 것으로 사료된다. 호산구자멸사 과정에서 Bcl-2 family와 caspase에 대한 후속 연구가 필요하다.

핵심 되는 말: CD30, 호산구, 자멸사, Bcl-2, caspase, 알레르기