CD30 supports lung inflammation

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Abstract

The physiological functions of CD30 have not been fully elucidated. Here we show that in CD30-deficient mice (CD30$^{-/-}$), lung inflammation is significantly diminished in the ovalbumin (OVA) model of airway hyperreactivity. In CD30$^{-/-}$ mice, the recruitment of eosinophils into the airways after OVA–aerosol challenge of OVA-primed mice was significantly diminished when compared with wild-type (w.t.) mice. IL-13 levels were also significantly reduced in CD30$^{-/-}$ mice while levels of IFN-γ, IL-4, IL-5 and IgE in bronchoalveolar lavage fluid, lung tissue and serum were comparable to w.t. mice. Peribronchial lymph node cells from CD30$^{-/-}$ mice, re-stimulated in vitro with OVA, secreted significantly lower levels of IL-13 than those from w.t. mice, but showed normal proliferative response and other cytokine production. Exogenous IL-13 reconstituted airway recruitment of leukocytes in OVA-challenged CD3O$^{-/-}$ mice. Adoptive transfer to naive w.t. mice of in vitro OVA-re-stimulated spleen cells from CD30$^{-/-}$ mice failed to induce eosinophilic pulmonary inflammation in contrast to transfer of primed cells from w.t. mice. These results indicate that CD30 is a regulator of T_h2 responses in the effector–memory phase and a regulator of IL-13 production in memory cells in the lung.

Introduction

Since the first identification on Hodgkin's Reed–Sternberg cells (1), CD30 has been studied extensively in its role in the pathogenesis of lymphomas (2, 3), its prognostic significance (4, 5) and its potential as therapeutic target (6–10). CD30 is also expressed on normal activated T cells (11–13), B cells (11, 13), macrophages (14) and NK cells (15). Activation-induced expression implicates CD30 as one of the regulators of the ensuing immune response. As a member of the tumor necrosis factor receptor superfamily (9, 16), CD30 co-stimulates cell proliferation and cell death. However, no consensus has been established regarding the pathophysiological role of CD30 in the immune system. Antibody production of murine B cells and isotype switching was enhanced by CD30 ligation (17), whereas it was not effective on human B cells (18).

Several lines of evidence support the hypothesis that CD30 plays a role in the T_h2 response. CD30 is expressed on CD4 T_h2 clones (19) but not on committed T_h1 clones (20). T_h2-related diseases are associated with higher CD30 expression in tissues or higher levels of soluble CD30 in serum (4, 21) and CD30 signals can induce T_h2 differentiation in vitro (19). CD30 expression is up-regulated by IL-4 (20) and CD28 signals (22) and down-regulated by IFN-γ and IL-12 (20), in an analogous way as T_h2 differentiation. However, no results have been reported to reveal a physiological role of CD30 in T_h2 responses in vivo in CD30$^{-/-}$ mice that have been found to have a partial defect in thymic negative selection (23).

One of us recently reported that CD30 signals on T cells can induce IL-13 production in vitro, independent of TCR signals, in a TRAF2/p38-mitogen-activating protein (MAP) kinase-dependent signaling pathway (24). A role for CD30 in the pathogenesis of allergic asthma has recently been reported but authors did not distinguish between priming and effector function (25). IL-13 production by CD30-expressing Hodgkin lymphoma cells has also been noted, suggesting a role for CD30 in the pathogenesis of lymphomas via IL-13 secretion (26). IL-13 is a T_h2 cytokine produced by T_h2-polarized cells.

IL-13 is the signature cytokine in lung inflammation and asthma (27–29). IL-13 recruits inflammatory cells into the lung via induction of vascular adhesion molecules and expression of numerous chemokines (30–32). IL-13 also supports subepithelial fibrosis and airway remodeling through IL-13 receptors on respiratory epithelial cells producing

TGF-β and through the recruitment, activation and induction of TGF-β in monocytes/macrophages (33–35). Metalloproteinases are induced by IL-13 and can activate latent TGF-β. Myofibroblasts respond to IL-13 with proliferation in a STAT-6-dependent process contributing to airway remodeling. IL-13 stimulates mucus hypersecretion in airway epithelial cells via induction of hCLCA1 or mCLCA3, putative Ca-activated chloride channels (36–38) and via induction of 5-lipoxygenase (39). Finally, IL-13 may be indirectly involved in mediating airway hyperreactivity (AHR) via its effects on epithelial cells; in addition, IL-13 may directly affect airway smooth muscle (ASM) cells since they express IL-4Rα1, IL-13Rα1 and IL-13Rα2 but not the common γ chain. The precise mechanisms by which IL-13 mediates its effects on ASM are still under investigation.

Asthma and pulmonary inflammation is usually driven by T_h2 responses inducing airway eosinophilia, mucus hypersecretion and AHR (40, 41). While IL-4 and IL-5 play important accessory roles in T_h2 polarization and eosinophilia during allergic lung inflammation, IL-13 has emerged as the primary cytokine responsible for lung inflammation (33, 42, 43). To gain insight into the functional role of CD30 in T_h2 responses *in vivo*, we employed a murine model of pulmonary inflammation using CD30$^{-/-}$ mice. Here, we present evidence indicating the participation of CD30 in a T_h2 response *in vivo*, contributing to IL-13 production and inflammation in the airways and to the recruitment of eosinophils.

Methods

Mice

CD30$^{-/-}$ mice on C57BL/6 background have been described by Amakawa *et al.* (23) and were generously provided T. Mak (Toronto, Canada). Wild-type (w.t.) C57BL/6 mice between 6 and 8 weeks of age were supplied from the Korean branch of Taconic, SamTaco (Osan, Korea).

Sensitization and challenge of mice

Mice were sensitized and challenged essentially as described by Kung *et al.* (44). Briefly, mice were sensitized with 10 μg of ovalbumin (OVA) (Sigma Chemical Co., St Louis, MO, USA) and 1 mg of alum intraperitoneally (i.p.) on days 0 and 5. Sham-immunized mice received alum alone. On day 12, mice were challenged by exposure to an aerosol of 1% OVA in PBS for 1 h following a 15-min chamber equilibration. Nebulization was achieved by the Schuco 2000 (Allied Health Care Products, St Louis, MO, USA) with a flow rate of 6 l min^{-1} at the nebulizer cup yielding particle sizes within 0.5–4.0 μm. A control group was exposed to saline alone with no OVA in an identical fashion.

Collection of serum, bronchoalveolar lavage fluid and lung homogenate

Three days after aerosol challenge, mice were anesthetized by i.p. injection of Avertin (2.5% w/v in PBS) and blood was taken by cardiac puncture. For bronchoalveolar lavage, the trachea was cannulated and the lungs were lavaged with three 0.4-ml aliquots of PBS. A part of the left lung was homogenized with PBS (0.8 ml of PBS for 100 mg of lung) us-

ing a T 10 basic homogenizer (Ika Works, Staufen, Germany). Homogenates were then centrifuged at 4°C (1200 × *g* for 5 min) and the supernatants stored at 20°C until the cytokine assays were performed.

Lymph node cell isolation

Peribronchial lymph nodes (LNs) were obtained by dissection and placed in chilled Roswell Park Memorial Institute 1640 medium (Life Technologies, Gaithersburg, MD). LN cells were harvested on day 15. Adherent cells were removed by adherence to plastic dishes and T cells were prepared by negative selection using anti-B220 and anti-NK cell (DX5)-coated magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany). Syngeneic B cell-enriched splenocytes were used as antigen-presenting cells (APCs) that were prepared by plastic attachment and subsequent treatment with mitomycin C for 20 min. T cells (5 × 10^5 cells) together with 5 × 10^5 APCs were cultured with OVA (200 μg ml^{-1}) in complete Iscove's Modified Dulbecco's Medium (IMDM) medium supplemented with 10% Fetal Bovine Serum. Cultures were incubated for 3 days and supernatants were collected for cytokine analyses or cultures were pulsed with 1 μCi of [^{3}H]thymidine per well. After incubation at 37°C for 18 h, triplicate wells were harvested onto glass fiber filters and incorporated radioactivity was measured in a scintillation counter. The background proliferation in the absence of added OVA was subtracted from the results.

Intratracheal instillation of murine recombinant IL-13

Mice were anesthetized and intubated with an angled 25-gauge needle. Afterward, 2 μg recombinant murine rIL-13 (PeproTech, Rocky Hill, NJ, USA) dissolved in 50 μl PBS (or 50 μl PBS only for control mice) was instilled everyday after aerosol challenge for three consecutive days. Analysis of bronchoalveolar lavage fluid (BALF) was performed 2 days after last IL-13 administration as previously described (45).

Isolation and adoptive transfer of splenocytes

Induction of lung inflammation with adoptively transferred lymphocytes from w.t. and CD30$^{-/-}$ mice was assessed as described previously. Briefly, 7 days after second immunization, the splenocytes were re-suspended at 2.5 × 10^6 cells ml^{-1} in complete IMDM medium and cultured for 3 days at 37°C in the presence of 200 μg ml^{-1} OVA. After washing, 1 × 10^7 cells re-suspended in 100 μl sterile saline were injected i.p. into w.t. recipient mice. Seven days later, recipient mice were aerosol challenged as described above and then lung inflammation was evaluated.

Cytokine assays (ELISA)

Protein levels of IL-2, IL-4, IL-5, IL-13 and IFN-γ in BALF and culture supernatants and IgE in serum were determined by ELISA according to the manufacturer's recommendation. Antibody pairs and standards were purchased from BD PharMingen (San Diego, CA, USA; IL-5), Biosource International (Camarillo, CA, USA; IL-2, IL-4, IFN-γ) or R&D Systems (Minneapolis, MN, USA; IL-13). The lower limits of detection for the cytokines were IL-2, 3 pg ml^{-1}; IL-4, 5 pg ml^{-1}; IL-5, 5 pg ml^{-1}; IFN-γ, 15 pg ml^{-1} and for the IL-13, 3 pg ml^{-1}.

Histology and eosinophil counting in the lung

Lung segments taken near the main bronchus were fixed in 10% neutral buffered formalin, pH 7.4 (Sigma-Aldrich, St Louis, MO, USA), and embedded in paraffin and 4-µm tissue sections were deparaffinized and rehydrated. To demonstrate eosinophils, the sections were stained with hematoxylin-2 (Richard-Allan Scientific, Kalamazoo, MI, USA) for 5 s and after brief rinse in distilled water, immersed in alkaline sodium chloride solution (1.0% sodium hydroxide and saturated sodium chloride in 80% ethanol) for 20 min and then stained in 0.5% Congo red solution (0.5% Congo red in 50% ethanol) for 20 min and dehydrated and mounted (47). Eosinophils were counted by light microscopy by a single observer who was unaware of the identity of the individual sections. Eosinophils in the lung were counted in 4–6 randomly selected sections and the results were expressed as the mean ($\pm$SE) cells per mm^2.

RNA extraction and reverse transcription–PCR

Total RNA was extracted from lung tissue by using Trizol reagent (Life Technologies) as per protocol and cDNAs were synthesized using random primers. Gene-specific primers for PCR amplification are mouse CD30, 5′-CAA CCC TGG CTG AGT TAC TC-3′ and 5′-AGC GGC AGG TTC TCC AGG TA-3′ to generate a 850-bp product and mouse CD30 ligand (CD30-L), 5′-ATG AGG AGA GAT AAG ATG T-3′ and 5′-CAC GGA TTG AGG ACA TAA CC-3′ to generate a 700-bp product. The PCR was performed in a final volume of 50 ml containing 1 pmol of each primer, 2 ml of diluted cDNA and 1.25 U of DNA polymerase (Promega, Madison, WI, USA). The samples were amplified for 30 cycles at 94°C (1 min), 55°C (1 min) and 72°C (2 min). Five microliters of each sample was separated by 2% agarose gel electrophoresis, and DNA was visualized by ethidium bromide staining.

Flow cytometric analysis

Expression of CD30 and CD30-L on LN T cells was analyzed by two-color flow cytometry. The cells were incubated with FITC- or PE-conjugated anti-CD4 or anti-CD8 and then FITC-conjugated anti-CD30 or PE-conjugated anti-CD30-L (Pharmingen) at 4°C for 30 min. After washing three times, stained cells were analyzed with the FACScan (Becton Dickinson Co.) and the Lysis II program.

Statistical analysis

Statistical analysis and graphical presentation were done using SigmaPlot 5.0 (SPSS Inc.). Values are given as mean $\pm$ SE and group means were compared with Student's *t* test in which $P < 0.05$ was considered significant.

Results

Diminished allergic airway inflammation in CD30$^{-/-}$ mice

OVA–aerosol challenge of mice primed with OVA is known to provoke a significant increase in eosinophil infiltration in lung tissues (Fig. 1a–d) and in BALF (Fig. 1e). OVA-primed CD30$^{-/-}$ and w.t. mice had normal lung histology and cell composition in BALF after PBS–aerosol (control) challenge (Fig. 1). After OVA–aerosol challenge, the lungs of w.t. mice

showed strong leukocyte/eosinophil infiltration while the lungs of CD30$^{-/-}$ mice showed significantly ($P = 0.01$) reduced infiltration by histopathology (Fig. 1, Table 1). In the BALF of CD30$^{-/-}$ mice, the total number of leukocytes was significantly lower than in w.t. mice ($P = 0.002$) (Fig. 1e). In differential counts, the numbers of lymphocytes, neutrophils and particularly eosinophils were markedly reduced in CD30$^{-/-}$ mice in comparison to w.t., while the number of macrophages was comparable between the two groups (Fig. 1e).

CD30 and CD30-L are expressed in vivo following antigen challenge

To independently verify the functional role of CD30 signals during lung inflammation, we measured CD30 and CD30-L expression in lung-associated lymphocytes after OVA–aerosol challenge of OVA-primed and non-immunized mice. The major populations of cells expressing CD30 and CD30-L in bronchial LNs were CD3$^+$ T cells (Fig. 2A and B). CD4$^+$ and CD8$^+$ T cells showed similar levels of CD30 expression,
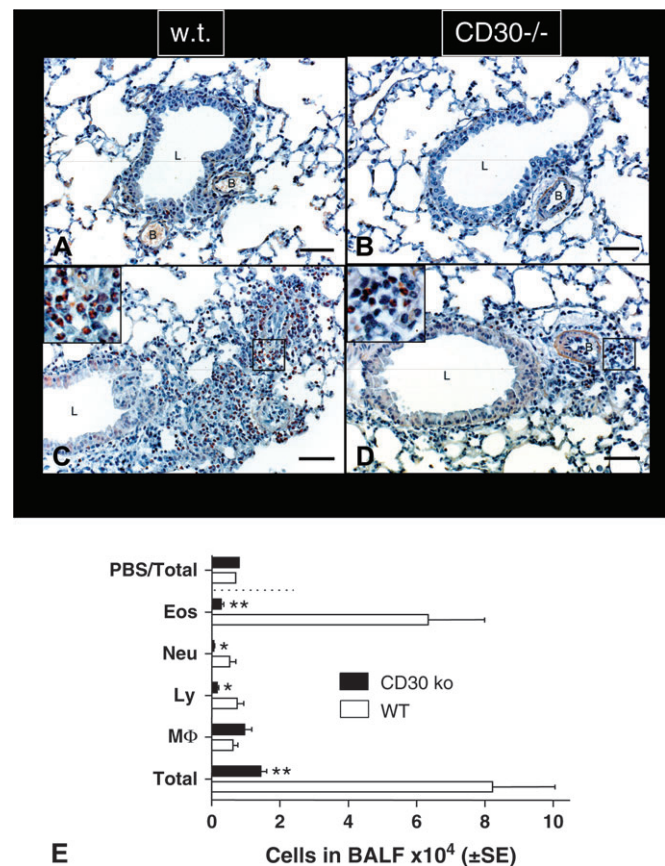
Fig. 1. Diminished leukocyte airway infiltration in CD30$^{-/-}$ mice. W.t. and CD30$^{-/-}$ mice were sensitized with OVA on days 0 and 5 as described and challenged by exposure to OVA aerosol on day 12. Three days later (on day 15), mice were assessed by histopathology. (A and C) w.t.; (B and D) CD30$^{-/-}$. Upper panels: airway challenge with PBS; lower panels: challenge with OVA. Sections are stained with hematoxyin and Congo red to reveal eosinophils. (E) Cells in BALF after PBS (top) and OVA–aerosol challenge. Eos, eosinophils; Neu, neutrophils; Ly, lymphocytes; MF, macrophages/monocytes; L, lung bronchus; B, blood vessel; $n = 8$, four independent experiments; *$P < 0.025$; **$P < 0.0025$.

while CD30-L expression predominated in CD4^{+} cells. Prominent expression of CD30 and CD30-L was also observed in non-T cell populations in bronchial LN (Fig. 2A and B). Moreover, elevated expression of CD30 and CD30-L mRNA was

found by PCR also in lung tissues after aerosol challenge (Fig. 2C).

Table 1. Lung tissue eosinophils in CD30^{−/−} and CD30^{+/+} (w.t.) mice, challenged with OVA–aerosol or PBS

Challenge	Mice	No. of eosinophils per mm^2 (mean ± SEM)	*P* value
PBS	W.t.	1.25 ± 0.028	NS
PBS	CD30^{−/−}	1.305 ± 0.044	
OVA	W.t.	74.45 ± 6.406	0.011
OVA	CD30^{−/−}	49.78 ± 4.416	

NS, not significant. Total numbers of eosinophils per mm^2 of lung tissues are mean ± SEM. *$P < 0.05$ versus the corresponding CD30^{+/+} (w.t.) value.

### IL-13 cytokine levels are selectively depressed in vivo in CD30 deficiency

It has been reported previously that CD30 signals selectively stimulate IL-13 production *in vitro* via p38-MAP kinase signals in murine CD4+ T cells (24). To compare *in vivo* cytokine production of w.t. and CD30^{−/−} mice, we measured cytokine levels in BALF and in lung homogenates after OVA–aerosol challenge. As shown in Fig. 3, levels of IL-4, IL-5 and IFN-γ were not significantly different between w.t. and CD30^{−/−} mice. However, IL-13 levels of CD30^{−/−} mice were significantly lower in BALF and lung homogenates than those of w.t. mice (Fig. 3). IgE levels associated with airway inflammation were similar in CD30^{−/−} and w.t. mice in BALF, lung tissue and serum (Fig. 4).
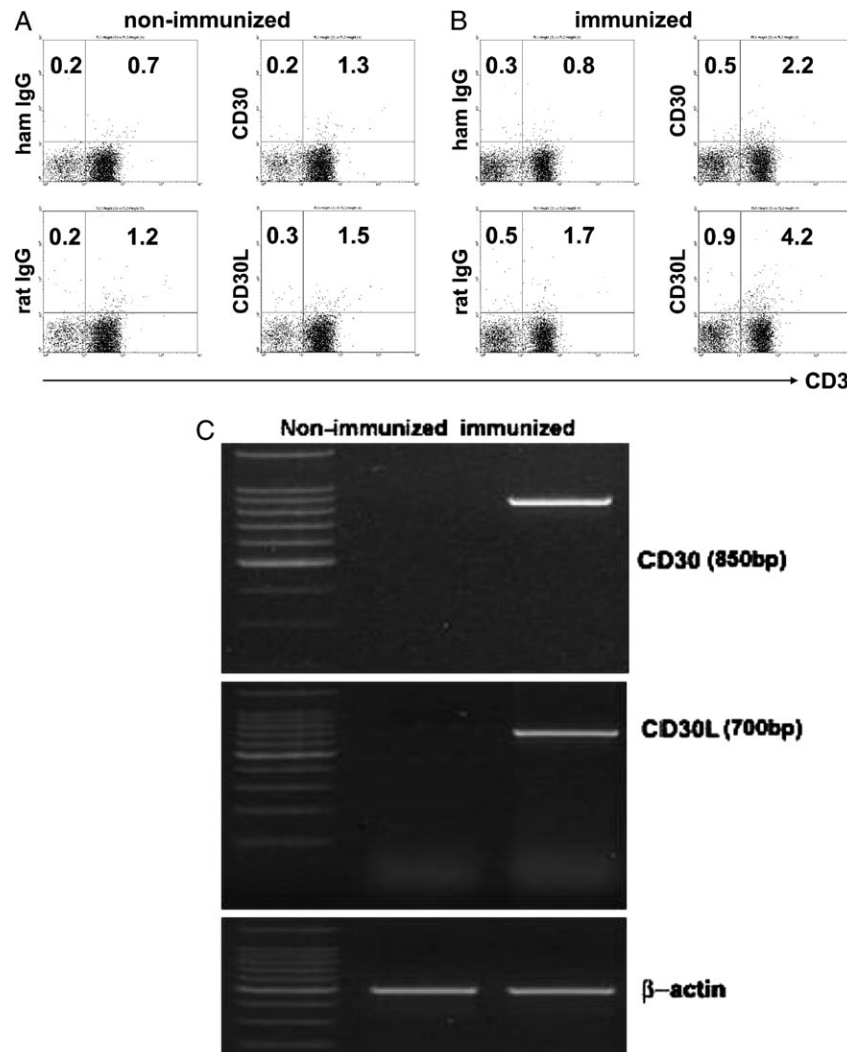
Fig. 2. Expression of CD30 and CD30-L in bronchial LNs of immunized and aerosol-challenged mice. W.t. mice were left unimmunized (A) or primed with OVA and then airway challenged (B). Bronchial LN cells were isolated and stained with FITC- or PE-conjugated anti-CD3 and then with FITC-conjugated anti-CD30 or PE-conjugated anti-CD30-L. As negative control, labeled hamster or rat IgG were used. Stained cells were analyzed by flow cytometry. (C) Expression of CD30 and CD30-L assessed by reverse transcription–PCR in lungs of mice with and without immunization and aerosol challenge.
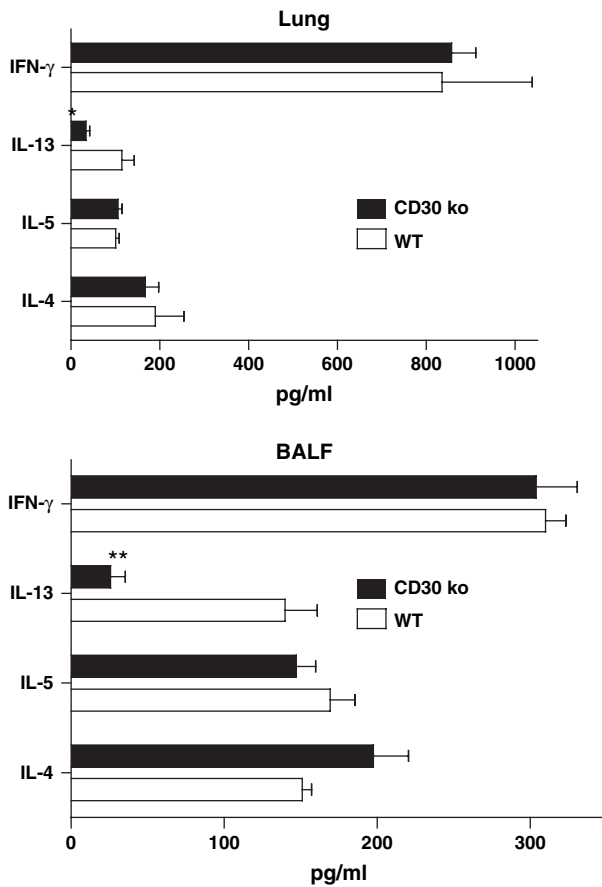
Fig. 3. Diminished IL-13 secretion in lungs and BALF of CD30$^{-/-}$ mice. W.t. and CD30$^{-/-}$ mice sensitized and airway-challenged with OVA were sacrificed on day 15 of the OVA protocol and BALF was obtained to determine BALF levels of cytokines. A part of the left lung was homogenized as described in Methods. Cytokine levels in BALF and the supernatants of lung homogenates were determined by ELISA; *n* = 9, three or four (IL-4) independent experiments; *$P < 0.05$; **$P < 0.005$.
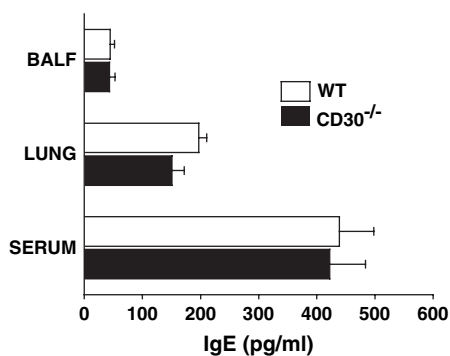
Fig. 4. IgE levels are not diminished in CD30$^{-/-}$ mice. W.t. and CD30$^{-/-}$ mice were sacrificed on day 15 of the OVA protocol and serum was prepared from the blood taken by cardiac puncture. BALF and lung homogenates were obtained as described. IgE levels in BALF, the supernatants of lung homogenates and serum were determined by ELISA. Data represent the mean ± SEM for groups of 4–5 mice.

Draining bronchial LN cells from OVA-primed w.t. and CD30$^{-/-}$ mice re-challenged with OVA *in vitro* exhibited similar proliferative responses (Fig. 5a) and produced comparable cytokine levels (Fig. 5b). LN cells from w.t. and CD30$^{-/-}$ mice labeled with carboxyfluorescein succinimidyl ester (CFSE) also showed similar moderate dilution (data not shown). However, IL-13 production of re-stimulated LN cells of CD30$^{-/-}$ mice was significantly impaired in comparison to w.t. (Fig. 5b).

*Exogenous IL-13 partially restores airway recruitment of eosinophils in CD30$^{-/-}$ mice*

CD30$^{-/-}$ mice may have diminished responsiveness to IL-13. To exclude this possibility, we reconstituted local IL-13 by instillation of rIL-13 into the airways of w.t. and CD30$^{-/-}$ mice. Instillation of exogenous IL-13 alone without antigen challenge induced airway recruitment of leukocytes and eosinophils in both w.t. (45) and CD30$^{-/-}$ mice (Fig. 6). In OVA–aerosol-challenged CD30$^{-/-}$ mice, exogenous IL-13 largely restored airway recruitment of eosinophils albeit not to the same levels found in primed w.t. mice plus IL-13 (Fig. 6).

CD30 signals are required in the effector phase of lung inflammation

OVA-specific T$_h$2-polarized CD4 cells are able to adoptively transfer lung inflammation to naive recipients upon OVA airway challenge. We were interested to determine whether T$_h$2-polarized CD30$^{-/-}$ cells had the same pathogenic
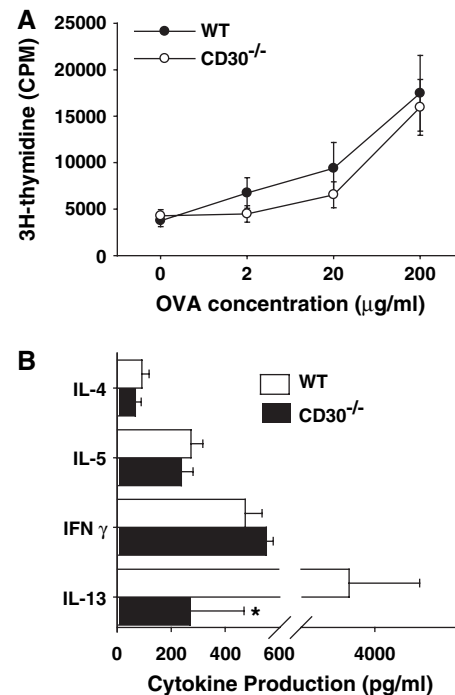
Fig. 5. Bronchial LNs of CD30$^{-/-}$ mice produce diminished IL-13 upon *in vitro* re-challenge with OVA. W.t. and CD30$^{-/-}$ mice were sacrificed on day 15 of the OVA protocol and LN cells obtained from peribronchial LNs were *in vitro* re-stimulated with OVA at 0–200 μg ml^{-1}. (A) Proliferation was measured by thymidine incorporation during the final 18 h of a 3-day antigen stimulation period. (B) Cytokines were measured by ELISA in supernatants obtained after a 3-day stimulation period of bronchial LN cells with 200 μg ml^{-1} OVA; *n* = 4–6, three independent experiments; *$P < 0.01$.

A   **Total leukocytes**

B   **Eosinophils**


Cells in BALF (x10³)
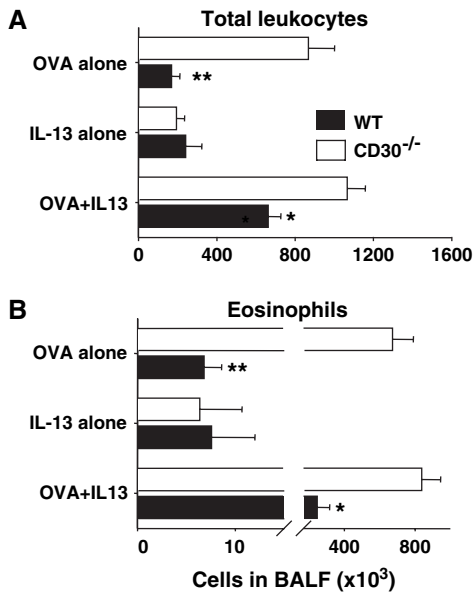
Fig. 6. Intratracheal instillation of recombinant IL-13 restores eosinophil infiltration in CD30$^{-/-}$ mice after primary immunization and airway challenge. IL-13 (2 μg in 50 μl PBS) was given intratracheally on three consecutive days either without prior OVA immunization (IL-13 alone) or after immunization and OVA airway challenge (OVA + IL-13) (on days 12, 13 and 14 of the OVA protocol). OVA alone indicates standard immunization and airway challenge; $n = 3$, two independent experiments; $*P < 0.05$; $**P < 0.01$.

potential. W.t. and CD30$^{-/-}$ mice were immunized i.p. with OVA and alum and their spleen cells re-stimulated with OVA *in vitro*. They were then compared in their ability to produce cytokines *in vitro* or to induce lung inflammation after adoptive transfer into naive w.t. mice. Spleen cells obtained from *in vivo* immunized CD30$^{-/-}$ and w.t. mice showed similar proliferation and cytokine production including IL-13 (Fig. 7A and B) after *in vitro* re-stimulation with OVA. These data indicate that CD30 signals are not essential for IL-13 production or T$_h$2 polarization by primary immunization *in vivo* and re-stimulation *in vitro*. However, in contrast to the transfer of w.t. cells to non-immune mice, adoptive transfer of OVA-specific, T$_h$2-polarized CD30$^{-/-}$ spleen cells failed to induce lung inflammation after OVA–aerosol challenge (Fig. 7C). This was not due to a lack of recruitment in CD30$^{-/-}$ mice. Similar numbers of adoptively transferred CFSE-labeled T cells were found in the lungs and BALF in CD30$^{-/-}$ and w.t. mice (data not shown). The data thus indicate CD30 signals elicited in the lung environment contribute to lung inflammation during the effector phase.

Discussion

Our data suggest that CD30 signals contribute to the T$_h$2 memory response responsible for lung inflammation in the murine OVA model. CD30$^{-/-}$ mice exhibit much less lung inflammation and IL-13 production in the lung after aerosol challenge than w.t. mice under comparable conditions.

A   **Changes in Cell Number**

B   **Cytokine Production**

C   **BALF Analysis**
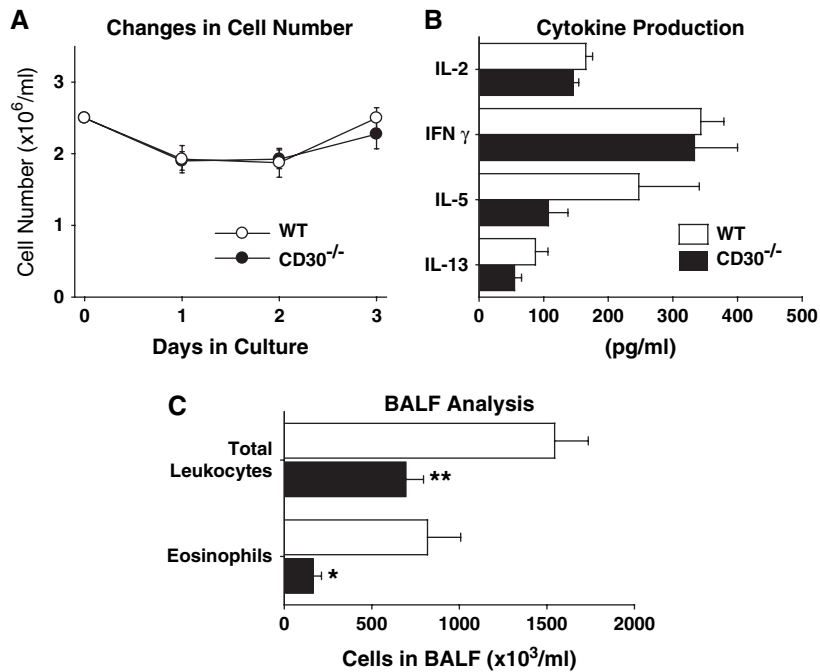
Fig. 7. OVA-specific CD30$^{-/-}$ cells do not transfer lung inflammation. (A) Cell number: mice were immunized i.p. twice with OVA and 7 days later the splenocytes were isolated and re-stimulated *in vitro* (2.5×10^6 ml^{-1}) with 200 μg ml^{-1} OVA. (B) Cytokines: supernatants were obtained after 3 days *in vitro* re-stimulation and cytokine titers analyzed by ELISA. (C) Adoptive transfer: 10^7 splenocytes, activated *in vivo* and re-stimulated *in vitro* with OVA, were adoptively transferred to naive w.t. mice and the mice challenged 7 days later with OVA aerosol; $n = 4$, two independent experiments; $*P < 0.05$; $**P < 0.01$.

These data are in agreement with reports by us and by others that CD30 signals on CD4 cells can induce IL-13 production even in the absence of TCR signals. Our data are the first to show that upon airway antigen challenge, CD30 signals enhance lung inflammation accompanied by increased IL-13 production of previously activated and T_h2-polarized effector cells while CD30 signals during primary immunization are not required for T_h2 polarization. Naive T cells express no CD30, and even after primary activation, CD30 expression is transient and apparently without consequence on polarization. However, T_h2-polarized cells upon adoptive transfer into w.t. mice need to receive CD30 signals in the lung in order to promote lung inflammation and IL-13 production. Apparently, T_h2-polarized, antigen-specific cells interact with CD30-L-expressing cells in bronchial draining LNs or in the lung parenchyma and induce lung inflammation and IL-13 production. In agreement with this, ongoing studies indicate that CD4 memory cells upon re-stimulation express high levels of CD30 (data not shown).

IL-13 production by both NK T cells and antigen-specific CD4 cells is necessary for induction of lung inflammation and AHR as reported by Akbari *et al.* (48). Initial production of IL-13 by NK T cells has been shown to be required as trigger for subsequently amplified IL-13 production by CD4 cells and lung inflammation. We have shown previously that CD4 cells can be triggered by CD30-L–CD30 interaction to synthesize IL-13. A potential role for CD30 on NK T cells is possible but has not so far been investigated.

CD30 expressed by CD4$^+$CD25$^+$ regulatory cells has been reported to support suppression of allograft rejection by CD8 memory cells (49). CD30 conferred to antigen-specific Tr cells the ability to induce apoptosis in CD8 memory cells. It is possible that the absence of CD30 on Tr cells may be able to down-regulate lung inflammation (47).

Other molecules contributing to lung inflammation in the OVA model have been described. OX40-L blockade results in reduced lung inflammation. Unlike our finding in CD30$^{-/-}$ mice, blockade of OX40-L during primary immunization prevents lung inflammation during secondary aerosol exposure. OX40-L co-stimulates T_h2 polarization and clonal expansion in primary activation. OX40-L blockade only during aerosol challenge has been reported in one publication as being ineffective for stopping lung inflammation (50). However, in a second publication, OX40-L blockade during aerosol challenge was found to also ameliorate lung inflammation due to reduction in the clonal expansion and apoptosis of OX40-positive memory CD4 cells (51).

Inducible co-stimulator (ICOS) and Inducible co-stimulator-ligand (ICOS-L) (B7-related protein) have been shown to be co-stimulators particularly for T_h2 responses. However, ICOS blockade during primary activation appears to aggravate allergic lung inflammation while ICOS blockade during aerosol exposure has little consequence on disease severity. This paradoxical effect of ICOS may be explained by the induction of Tr cells with the help of ICOS co-stimulation during primary activation (52–54).

It is clear that the molecular and cellular mechanisms of the induction and exacerbation of lung inflammation and asthma are complex and not fully understood. Our study points to a novel and unappreciated role of CD30 signaling in this important disease. Blockade of lung inflammation even in the presence of IL-13 production in BALF as seen in adoptive transfer experiments points to additional roles of CD30 in the regulation of lung inflammation. We favor the hypothesis that the absence of CD30 on adoptively transferred Tr cells during airway re-challenge may contribute to reduced lung inflammation. Further studies are under way to test this interesting possibility.

Abbreviations

AHR	airway hyperreactivity
APC	antigen-presenting cells
ASM	airway smooth muscle
BALF	bronchoalveolar lavage fluid
CFSE	carboxyfluorescein succinimidyl ester
CD30$^{-/-}$	CD30-deficient mice
CD30-L	CD30 ligand
IMDM	iscove's modified dulbecco's medium
i.p.	intraperitoneal
LN	lymph node
MAP	mitogen-activating protein
OVA	ovalbumin
w.t.	wild type

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