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Differential rates of apoptosis and recruitment limit eosinophil accumulation in the lungs of asthma-resistant CBA/Ca mice

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ABSTRACT

The accumulation of eosinophils is a common feature of allergic airway inflammation and correlates with disease severity. In an ovalbumin (OVA)-induced murine model of allergic lung disease, CBA/Ca mice develop much lower levels of lung eosinophilia, lung oedema, mucus hypersecretion and airways obstruction than BALB/c and C57BL/6 strains. In this study these strains have been examined to identify mechanisms that control the recruitment and survival of eosinophils in the allergic lung. Following immunization with OVA, CBA/Ca mice developed a robust systemic allergic response, with high levels of total and OVA-specific IgE and increases in peripheral blood eosinophils. Lung eotaxin-1 levels and expression of CD18 on eosinophils recovered by bronchoalveolar lavage (BAL) were least pronounced in CBA/Ca mice, whereas mRNA for L-selectin was highest in eosinophils from C57BL/6 mice. Apoptosis of BAL eosinophils *ex vivo* was most pronounced in the CBA/Ca strain. BALB/c mice expressed the highest levels of the eosinophil growth and survival factor interleukin (IL)-5 in the lungs and BAL eosinophils from these animals expressed more of the anti-apoptotic proteins Bcl-x_L and Bcl-2 than cells from the other strains. A combination of lower levels of recruitment and rapid apoptosis may therefore limit the accumulation of eosinophils and pathology in the lungs of CBA/Ca mice. In addition, although the level of pathology that developed in C57BL/6 and BALB/c mice was similar, some of the underlying mechanisms are likely to differ.

1. Introduction

Asthma is characterised by airway inflammation, reversible obstruction of airflow and chronic structural changes. The late phase of the asthmatic response to an allergen involves recruitment and persistence of inflammatory leukocytes and eosinophils can be a prominent feature. Levels of circulating eosinophils are increased in atopic individuals and correlate with asthma severity (Takenaka et al., 1975). Eosinophils may contribute to acute inflammation in asthma and may also be involved in chronic remodeling of tissues (Flood-Page et al., 2003). Mice deficient in eosinophils do not develop airways hyperreactivity (AHR) (Foster et al., 1996; Lee et al., 2004), mucus hypersecretion, airways obstruction (Lee et al., 2004), collagen deposition and smooth muscle hyperplasia (Humbles et al., 2004).

Eosinophil recruitment is dependent on a combination of cytokines, chemokines and adhesion molecules that control selective migration across vascular endothelium. Eotaxin-1 is an eosinophil-specific chemoattractant (Jose et al., 1994; Rothenberg et al., 1995), involved in recruitment from the bone marrow into the blood (Lopez et al., 1988) and to inflammatory sites (Ganzalo et al., 1996; Rothenberg et al., 1996). The transmigration of eosinophils across the endothelium is also dependent on interactions between integrins on eosinophils and intercellular adhesion molecules expressed by endothelial cells (Lo et al., 1989; Smith et al., 1989; Dobrina et al., 1991; Weller et al., 1991; Abraham et al., 1994; Pretolani et al., 1994; Ohkawara et al., 1995; Gonzalo et al., 1996; Kitayama et al., 1997). Eosinophils express E- and P-selectin ligands (Resnick and Weller, 1993), L-selectin (CD62L) (Lewinsohn et al., 1987; Resnick and Weller, 1993), high levels of β1-integrins, including very late antigen 4 (VLA-4) (Dobrina et al., 1991; Weller et al., 1991) and β2-integrins such as Mac-1 (CD11b/CD18) (Kimani et al., 1988) and lymphocyte function antigen 1 (LFA-1 or CD11a/CD18) (Hartnell et al., 1990).

The accumulation of eosinophils at inflammatory sites is dependent on both recruitment of these cells and factors allowing them to persist in the tissue. Caspase activation and programmed cell death or apoptosis can be induced in granulocytes by the

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intrinsic or mitochondrial pathway. Growth factor withdrawal or stress conditions cause variation in the expression and location of Bcl-2 family proteins including the anti-apoptotic proteins Bcl-2 and Bcl-x_L and the pro-apoptotic BH3-domain only proteins Bim and Bmf. Interleukin (IL)-5 is a growth, differentiation and survival factor for eosinophils and levels are increased in the lungs of asthmatic subjects, as is the frequency of Bcl-2^+ eosinophils (Jang et al., 2000). The extrinsic or receptor-mediated pathway of apoptosis and Fas have also been implicated in the apoptosis of human and mouse eosinophils (Matsumoto et al., 1995; Tsuyuki et al., 1995; Druilhe et al., 1996), but factors that control eosinophil survival have not been fully defined.

Mouse models of asthma are used to dissect the complex pathways underlying the disease. Common mouse strains differ both in propensity to develop allergen-induced inflammatory lung disease and in the mechanisms underlying pathology (Corry et al., 1996; Foster et al., 1996; Rankin et al., 1996; Zhang et al., 1997; Herz et al., 1998; Takeda et al., 2001; Whitehead et al., 2003). We have recently shown that the CBA/Ca mouse strain, even when expressing an IL-5 transgene, is relatively resistant to development of airway pathology in an acute model of allergic asthma (Dent, 2002; Tumes et al., 2007). CBA/Ca mice develop lower levels of airways obstruction, mucus production, lung oedema and eosinophil degranulation when compared to BALB/c and C57BL/6 mice. Additionally, CBA/Ca mice have markedly fewer eosinophils recoverable from the lungs following allergen challenge. We have found that eosinophils recovered from the lungs of ovalbumin (OVA)-challenged CBA/Ca mice are prone to undergo apoptosis and this may be a mechanism through which eosinophil numbers are regulated and, as a consequence of this, pathology is limited (Tumes et al., 2007). In this study we further delineate mechanisms of eosinophil recruitment and survival in the lungs of allergen-challenged mice.

2. Materials and methods

2.1. Animals

Heterozygous CBA/Ca, BALB/c and C57BL/6 Tg5C2 IL-5 transgenic (Tg) mice containing approximately 49 copies of the transgene (Dent et al., 1990; Dent, 2002) and wildtype (wt) littermates were bred under clean barrier conditions in the University of Adelaide Medical School Animal House. Experimental animals were fed and watered *ad libitum* and housed in clean conventional conditions under a controlled cycle of 12 h light. All animals were handled according to University of Adelaide Animal Ethics Committee guidelines.

2.2. Immunization and challenge

Mice were immunized and challenged as previously described (Tumes et al., 2007). Briefly, mice were immunized by intraperitoneal injection of 10 μg chicken egg ovalbumin (OVA) mixed with 1 mg of aluminum hydroxide gel in 50 μL of endotoxin free (ef) phosphate buffered saline (PBS), on each of days 0 and 12. Using a clinical nebuliser, mice were then challenged with aerosols of 1% OVA in efPBS or with efPBS alone, for either 20 min on each of days 28 and 29 (light challenge) or with three 30 min exposures on each of days 22, 24, 26 and 28 (heavy challenge). Mice were euthanized by CO_2 inhalation on day 30 or 32 (light challenge) or day 30 (heavy challenge).

2.3. Bronchoalveolar lavage and staining

Bronchoalveolar lavage (BAL) was performed with three aliquots of 1 mL PBS delivered via a cannula into the trachea (Tumes et al., 2007). Lavage cells were resuspended in PBS, counted using a hemocytometer and differential cell counts were performed on cytocentrifuged preparations stained with Giesma (BDH Laboratory Services, Poole, Dorset, UK).

2.4. Detection of leukocyte apoptosis

Annexin-V and propidium iodide (PI) staining were used to detect viable and apoptotic eosinophils from *in vitro* cultures as previously described (Tumes et al., 2007).

2.5. Quantitation of mRNA expression in eosinophils and whole lung tissue

To ensure high yield and purity, eosinophils were isolated by BAL from IL-5 Tg mice exposed to the heavy challenge regime. Eosinophils were sorted to ≥98% purity on the basis of distinctive forward scatter (FSC) and side scatter (SSC) properties using a Beckman Coulter Altra fluorescence-activated cell sorter and Expo32 MultiCOMP version 1.2B software (Beckman Coulter, Miami, FL, USA). Total RNA was extracted, purified and reverse transcribed as described previously (Tumes et al., 2007). For analysis of whole lung tissue, the left lung was removed and homogenised in 2 mL of lysis buffer (Invitrogen, Carlsbad, CA, USA) using an Ultra Turrax homogeniser (Janke and Kunkel, Staufen, Germany) for 60 s. RNA was then isolated using a PureLink Micro-to-Midi Total RNA Purification System (Invitrogen, Carlsbad, CA, USA) and DNase treated using Turbo DNA-free (Ambion, Austin TX, USA). 1.5 μg of RNA per 20 μl reaction was reverse transcribed using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Quantitative real time reverse transcriptase polymerase chain reaction (qRT-PCR) was performed on a Rotor Gene 2000 Real-Time Cycler and analysed using Rotor Gene, version 4.6 software (Corbett Research, Mortlake, NSW, Australia) using Power SYBR Green (Applied Biosystems, Warrington, Cheshire, UK). Porphobilinogen deaminase (PBGD) and 18S ribosomal RNA (18S RNA) were used as controls. Normalized intensity was calculated using qBASE (Hellemans et al., 2007). Primers (Geneworks, Adelaide, SA, Australia) used were: PBGD (sense: 5′ actgtggtggcgatgctg 3′, antisense: 5′ gcaaggtttccagggtctt 3′ GenBank/EMBL/DDBJ accession no. BC_003861; calculated product length, 303 bp), 18S rRNA (sense: 5′ gagaaacggctaccacatcc 3′, anti-sense: 5′ accagacttgccctccaa 3′; NR_003278; calculated product length, 169 bp), eotaxin-1 (sense: 5′ cacggtcacttccttcacct 3′, anti-sense: 5′ cttcttcttggggtcagcac 3′; NM_011330; calculated product length, 202 bp), L-selectin (sense: 5′ acgagactctgggaaatgga 3′, anti-sense: 5′ ccgtaataccctgcatcaca 3′; NM_011346; calculated product length, 156 bp).

2.6. Detection of IL-5 and apoptosis proteins

IL-5 concentrations were measured in BAL fluid (BALF) by ELISA with a sensitivity limit of 40 pg/mL (Tumes et al., 2007). To detect apoptosis proteins by western blotting, BAL cells (consisting of 90, 94 and 97% eosinophils for CBA/Ca, BALB/c and C57BL/6 donors, respectively) were recovered from IL-5 Tg mice following heavy OVA challenge. 2×10^6 cells were lysed in Laemmli buffer consisting of 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, 0.01% bromophenol blue, 62.5 mM Tris–HCl by vortexing and then heating to 100 °C for 10 min. Samples were then separated by SDS-PAGE on 10–15% polyacrylamide gels and transferred to nitrocellulose membranes. Controls used were B300 cells that had been grown beyond confluence and spleen cells that were cultured for 6 h in the presence of 1 μg/ml Jo2 anti-Fas antibody (BD Pharmingen, San Jose, CA, USA). Primary antibodies: Bcl-2, clone 3F11 (BD Pharmingen, San Jose, CA, USA), Bcl-x_L, clone 7B2.5 (Chemicon, Temecula, CA, USA), Caspase 3,

clone 19 (Transduction Laboratories, Lexington, KY, USA), Caspase 8 (kindly provided by Andreas Strasser) and control antibody β-actin, Clone AC-15 (Sigma–Aldrich Co., St. Louis, MO, USA) were incubated overnight at 4 °C followed by incubation with appropriate biotinylated secondary antibodies. Signal was detected following incubation with streptavidin–HRP conjugate and enhanced chemiluminescence (ECL) western blotting reagents (Amersham, Little Chalfont, Buckinghamshire, UK) and exposure to Ultra UV-G medical X ray film (AGFA, Morstel, Belgium). Images were acquired using a molecular imager FX and densitometry analysis performed using Quantity One version 4.0.3 (Biorad, Cambridge, MA, USA).

2.7. In vitro eosinophil chemotaxis

Eosinophils were recovered from the lungs of six wt mice/strain following a heavy challenge regime, pooled and 50 μL of cells suspension at 10^6 cells/mL were added into the upper wells of an AP48 Neuroprobe chamber containing an 8-μm pore membrane (Costar, Rochester, NY, USA). 27 μL of synthetic mouse eotaxin-1 at 500 ng/mL was added to the bottom wells and samples in quadruplicate were incubated for 2 h at 37 °C/5% CO_2. Cells that did not migrate were gently wiped from the membrane before methanol fixation and Giemsa staining (Merk, Darmstadt, Germany). Eosinophils were enumerated using light microscopy (1000× magnification) in 8 fields/well.

2.8. Statistical analysis

Statistical analysis was performed using either one and two-tailed Student's unpaired *t*-tests, or one way ANOVA using the Tukey post test, where appropriate. A confidence interval of 95% was used for all analyses. All statistical analyses were calculated using GraphPad Prism version 5.0.1 for Windows (GraphPad software, San Diego, CA, USA).

3. Results

3.1. Peripheral response to immunization and challenge

To determine if the relative absence of pathology in OVA-challenged CBA/Ca mice (Tumes et al., 2007) was due to anergy to allergen and/or a poor IgE response, wt mice of all three strains were assessed throughout an OVA immunization and heavy aerosol challenge protocol. There was a similar increase in eosinophils in the peripheral blood in response to immunization in all strains, which peaked at days 22 and 30, i.e. immediately before the first and 2 days after the last challenges (Fig. 1). By day 26 the pool of eosinophils in the blood had decreased (Fig. 1) and this was coincident with accumulation of eosinophils in the lungs (data not shown). All three strains showed significant increases in both total and OVA-specific serum IgE at day 17 of the immunization protocol (data not shown).

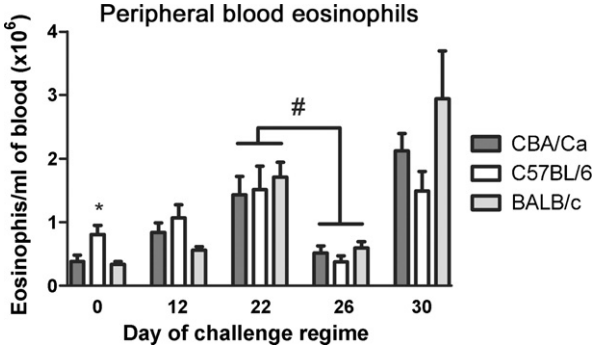

Fig. 1. Peripheral blood eosinophils in wt CBA/Ca, BALB/c and C57BL/6 mice during immunization and a heavy OVA aerosol challenge regime. Data expressed as mean + S.E.M., $n = 6–7$ mice/group. *indicates C57BL/6 > CBA/Ca and BALB/c ($p < 0.05$) and #Indicates D22 > D26 ($p < 0.05$).

3.2. Eosinophil recruitment

The number of BAL eosinophils was increased in all three wt strains following a light allergen challenge, however at both 24 and 72 h after the last aerosol treatment, BAL eosinophil numbers were lowest in CBA/Ca mice. A modest increase in the number of BAL eosinophils was evident 24 h following allergen challenge in all strains and by 72 h eosinophils had increased 5–10-fold in the asthma-susceptible BALB/c and C57BL6 strains and significantly less so in the CBA/Ca mice (Fig. 2A and B). Factors that might play a role in the accumulation of eosinophils in the lungs of these wt mice were investigated. In the light challenge regime, a significant increase in BALF IL-5 concentration was detected in OVA-challenged mice 24 h after exposure (Fig. 2C) and had decreased to levels comparable with PBS challenged controls by 72 h (Fig. 2D). Although wt CBA/Ca mice had lower levels of BAL IL-5 than the other strains, this did not reach statistical significance. However, BALF IL-5 concentrations in IL-5 Tg mice were significantly lower in samples from OVA-challenged CBA/Ca mice than for similarly treated animals from either of the other strains at both day 30 and 32 (e.g. for day 30, mean + S.E.M. for CBA/Ca, C57BL/6 and BALB/c: for OVA-challenged, 0.65 + 0.15*, 3.13 + 0.71, 4.35 + 1.15 ng/mL respectively, *less than other OVA-challenged groups, $p < 0.05$; for PBS-challenged, 0.18 + 0.03, 0.13 + 0.01, 0.29 + 0.26 ng/mL respectively). mRNA encoding eotaxin-1 was not elevated in lung tissue 24 h after OVA challenge (Fig. 2E), but after 72 h a significant up-regulation was seen in OVA-challenged groups from all three strains (Fig. 2F). The increase in eotaxin-1 expression was significantly less in the CBA/Ca strain than in BALB/c and C57BL/6 animals (Fig. 2F), and this coincided with peak BAL eosinophil recovery (Fig. 2B and unpublished). Migration of wt BAL eosinophils *in vitro* in response to the chemokine eotaxin-1 was similar for the three strains (Fig. 3A). Adhesion molecules are also important for the recruitment of eosinophils and expression may be modulated during inflammation. The expression of both subunits of the Mac-1 integrin (CD11b/CD18) on eosinophils was assessed by flow cytometry. CD11b expression was similar on wt BAL eosinophils from all three strains (data not shown), however the expression of CD18 was lowest on eosinophils from CBA/Ca mice (Fig. 3B). The expression of mRNA specific for VLA-4 and L-selectin in purified eosinophils was measured using qRT-PCR. Expression of VLA-4 mRNA by CBA/Ca eosinophils was comparable to cells recovered from the other strains (data not shown). BAL eosinophils from C57BL/6 mice expressed the highest levels of L-selectin (Fig. 3C).

3.3. Eosinophil apoptosis

BAL eosinophils from allergen-challenged wt CBA/Ca mice lost viability more rapidly in culture than cells from either C57BL/6 or BALB/c mice (Fig. 4A). Eosinophils in early apoptosis (annexin-V^+, propidium iodide$^-$) were most prevalent from 4 to 10 h after initiation of cultures in eosinophils from CBA/Ca mice (Fig. 4B). To further investigate mechanisms regulating eosinophil survival, expression of proteins involved in apoptosis were assessed. Since strain differences in sensitivity of wt BAL eosinophils to apoptosis (Fig. 4A and B) were consistent with our earlier work in IL-5 Tg mice (Tumes et al., 2007), transgenic donors were used when the recovery of large numbers of eosinophils of high purity was required (Fig. 4C, D and Fig. 5). Caspase 3 processing occurs immediately prior to DNA

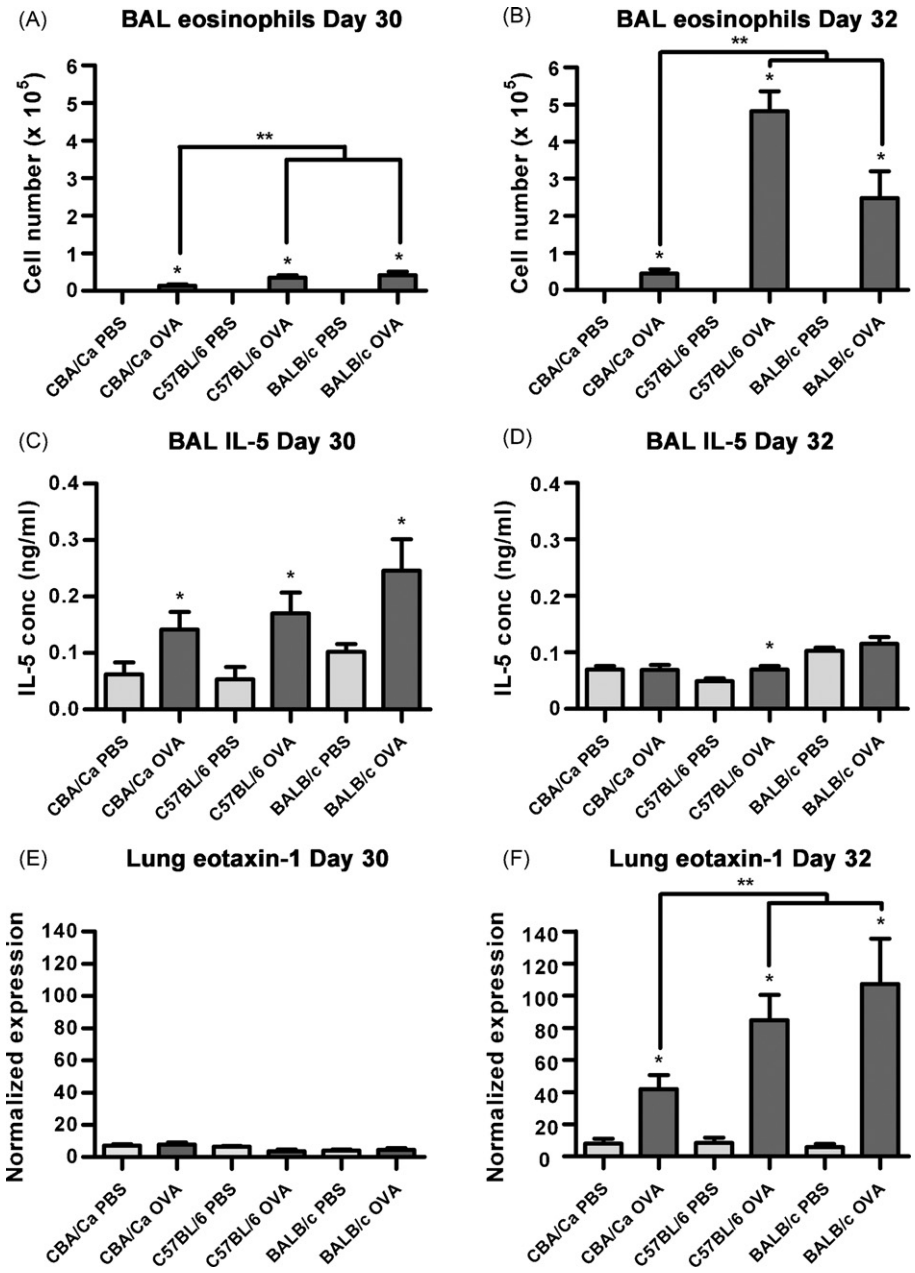

Fig. 2. Total BAL eosinophils (A and B), BALF IL-5 concentration (C and D) and quantitative RT-PCR analysis of eotaxin-1 mRNA expression in whole lung tissue (E and F) on day 30 (A, C and E) and day 32 (B, D and F) for wt mice subjected to a light OVA challenge regime. Eotaxin-1 normalized against PBGD and 18S rRNA levels. Data expressed as mean + S.E.M., $n = 7–8$ mice/group. *Indicates significantly greater than respective PBS-challenged control; **indicates CBA/Ca < C57BL/6, BALB/c ($p < 0.05$).

fragmentation and is involved in both extrinsic and intrinsic apoptosis. Marked caspase 3 processing was evident after 5 and 10 h of culture in BALB/c mice (Fig. 4C) and this persisted at lower levels at the 24 and 48 h time-points. Similar results were obtained from CBA/Ca and C57BL/6 mice (data not shown). Approximately 2% of BAL eosinophils from all three strains expressed Fas immediately following recovery (data not shown). Processing of caspase 8 is one of the initial events in the activation of apoptosis via the Fas and TNF-related family of receptors. Both the native (55 kDa) and processed (18 kDa) forms of caspase 8 were detected in spleen cells cultured with an anti-Fas antibody (Jo2) that induced apoptosis, but the 18 kDa activation fragment was not seen in lysates of BAL cells cultured for up to 48 h (Fig. 4D and data not shown). Addition of Jo2 did not increase the rate of apoptosis of BAL eosinophils from any of the strains (data not shown).

Collectively these data suggest that Fas and/or the TNF-related receptors are not involved in the apoptosis seen in cultured BAL eosinophils and so the intrinsic or mitochondrial cell death pathway was assessed. Immediately upon recovery and after 10 h of culture, BAL cells from OVA-challenged BALB/c mice expressed significantly higher amounts of the anti-apoptotic Bcl-2 and Bcl-x_L proteins than cells from C57BL/6 and CBA/Ca mice (Fig. 5A–E) and this strain difference was evident for at least 48 h (data not shown). The pro-apoptotic proteins Bim and Bmf can be important in the regulation of granulocyte survival (Villunger et al., 2003). Following OVA challenge mRNA for both of these genes was present in purified BAL eosinophils (data not shown). However while high levels of these proteins were detected in nutrient-depleted B300 cells used as a positive control, no Bmf and only very low levels of Bim_L protein were expressed in BAL cells from any of the strains (Fig. 5F).

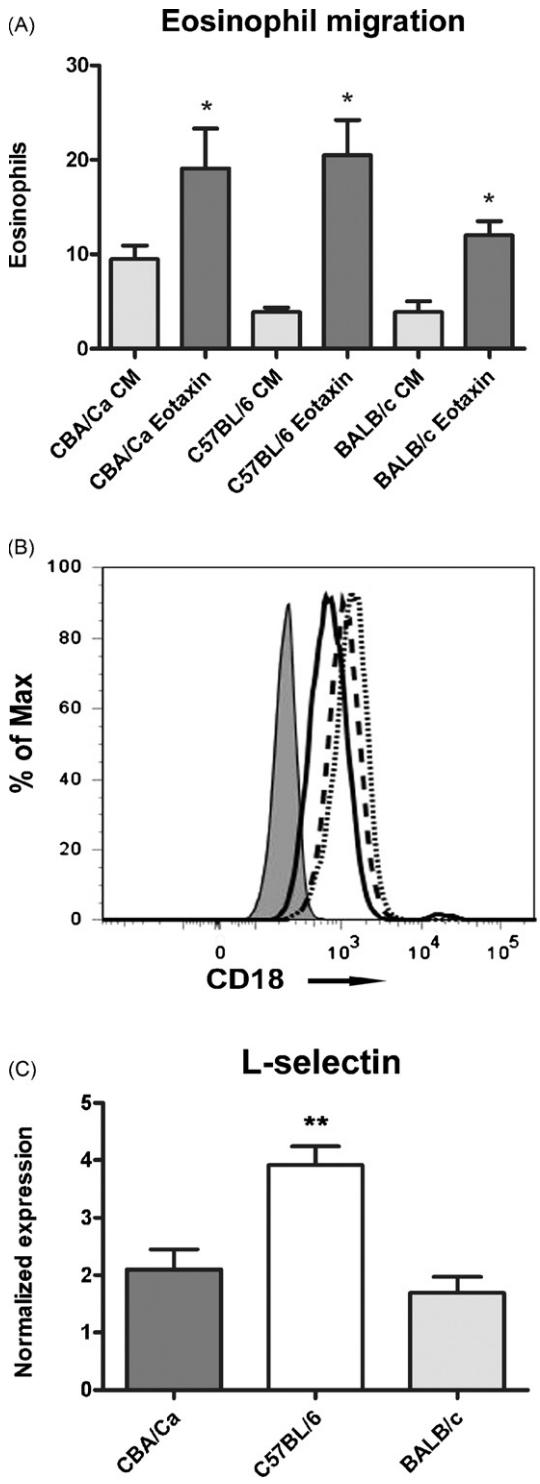

Fig. 3. Eosinophil migration *in vitro* (A) and surface expression of CD18 (B) using BAL cells from wt mice following heavy OVA challenge (6 mice/group). (A) Cells exposed to either 500 ng/mL synthetic mouse eotaxin-1 or culture medium (CM) alone ($n = 4$). (B) Shaded peak = negative control, solid line = CBA/Ca, dashed line = C57BL/6 and dotted line = BALB/c, data are representative of two separate experiments, a minimum of 10,000 events acquired/sample. (C) Quantitative RT-PCR analysis of L-selectin mRNA expression by purified BAL eosinophils from IL-5 Tg mice after heavy OVA challenge ($n = 7$–8 mice/group). Data expressed as mean + S.E.M. *Indicates significantly greater than respective CM only control ($p < 0.05$), **indicates C57BL/6 > BALB/c, CBA/Ca ($p < 0.05$).

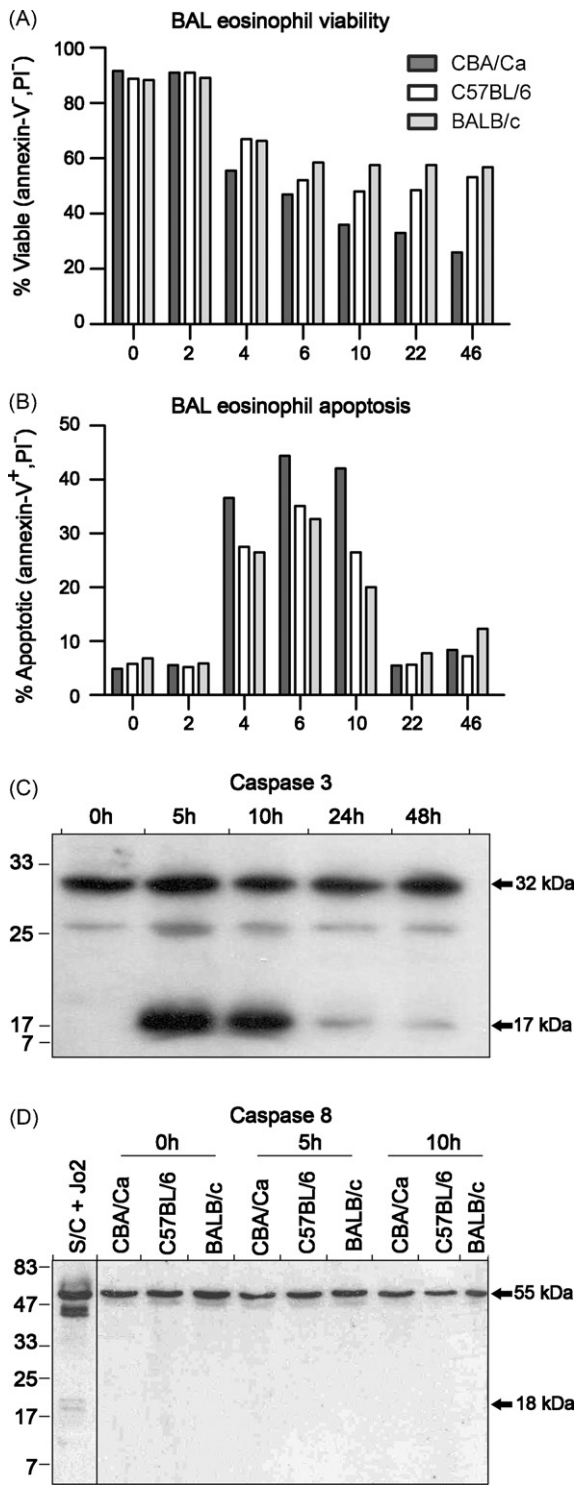

Fig. 4. Viability (A; annexin-V$^-$, PI$^-$) and early apoptosis (B; annexin-V$^+$, PI$^-$) of BAL eosinophils from wt mice after heavy OVA challenge regime. Cells pooled from 6 mice/strain, data expressed as mean for total of 10,000 events/sample for eosinophils selected on forward and side scatter flow cytometric characteristics, representative of three separate experiments. Western blot analysis for expression of caspase 3 (C) and caspase 8 (D) proteins by BAL eosinophils from IL-5 Tg BALB/c mice after heavy OVA challenge and culture for 0–48 h prior to lysis and analysis. (C) Caspase 3 expression in BAL cells after 0–48 h of culture, arrows indicate 32 kDa unprocessed form and 17 kDa activation fragment. (D) Caspase 8 expression after 0, 5 and 10 h culture, S/C + Jo2 = spleen cells incubated with anti-Fas antibody, arrows indicate the 55–58 kDa unprocessed form and the 18 kDa activation fragment of caspase 8.

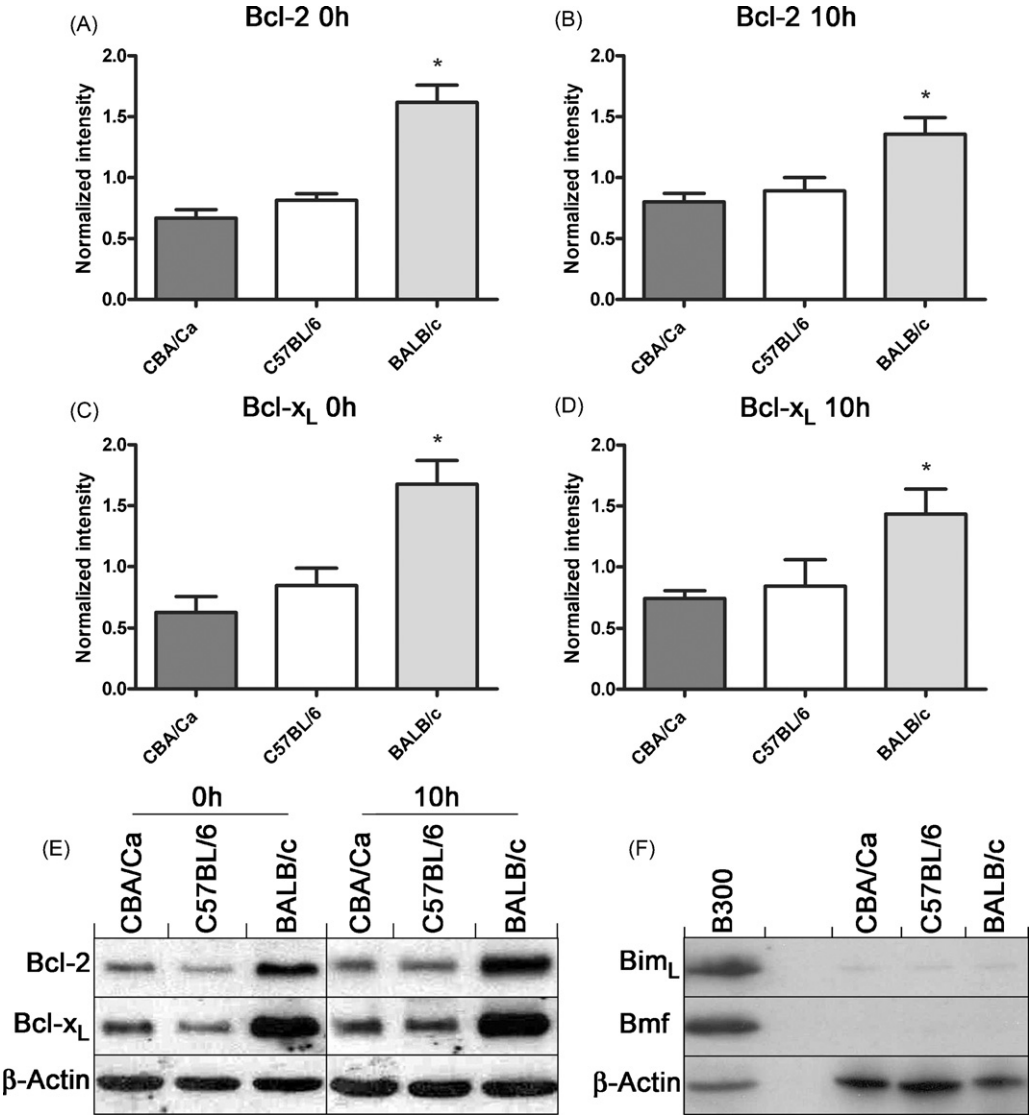

Fig. 5. Expression of Bcl-2 (A and B) and Bcl-x_L (C and D) in BAL cells from IL-5 Tg mice subjected to heavy OVA challenge, cells cultured for 0 h (A and C) and 10 h (B and D). Western blot analysis of expression, normalized against β-actin control and analysed by densitometry. Representative immunoblots (E) of Bcl-2, Bcl-x_L, and β-actin in BAL eosinophils pooled from 5 mice/group, cumulative data in A–D are from this and three other separate experiments. Expression of Bmf and Bim_L protein (F) by BAL cells sourced and treated as above. Data in A–D expressed as mean + S.E.M., *indicates BALB/c > C57BL/6, CBA/Ca ($p < 0.05$).

4. Discussion

We have previously shown that CBA/Ca mice are relatively resistant to development of pathology in acute models of experimental asthma (Dent, 2002; Tumes et al., 2007). In response to allergen challenge CBA/Ca mice develop much lower levels of lung eosinophilia than BALB/c and C57BL/6 strains. We have recently linked this phenomenon with a lung-specific propensity of eosinophils from the CBA/Ca strain to undergo apoptosis (Tumes et al., 2007). In this study we have further defined the mechanisms controlling allergen-induced accumulation of eosinophils in the lungs of these three strains of mice. We have detected strain-specific differences in expression of eotaxin-1, IL-5, adhesion molecules and anti-apoptotic proteins that may contribute to strain-specific variations in inflammatory processes. Table 1 summarizes the major differences in immunopathology seen in the three strains and potential underlying mechanisms.

Despite the limited pathology in the CBA/Ca mouse strain following immunization and challenge with OVA, these animals still develop an allergic response, characterised by elevated IgE and peripheral blood eosinophilia. The relatively modest accumulation of eosinophils in the airways and tissues of allergen-challenged CBA/Ca mice may be an important factor in limiting subsequent lung pathology and we have now demonstrated that this is not due to a deficiency in mobilization of eosinophils into the blood. Our data also suggest that eosinophilopoiesis in CBA/Ca mice is comparable to that in the other strains.

Eotaxin-1 is a potent chemoattractant for eosinophils (Jose et al., 1994; Rothenberg et al., 1995; Ponath et al., 1996; Rothenberg et al., 1996). Eosinophils from the three mouse strains express similar levels of mRNA for CCR3, the receptor for eotaxin-1 (Tumes et al., 2007) and in this study we have shown that there are no inter-strain differences in eotaxin-1 induced eosinophil migration *in vitro*. The time of peak eotaxin-1 production in murine asthma models varies in studies from different laboratories, possibly due to the timing and dose of allergen used (Rothenberg et al., 1995; Ganzalo et al., 1996; Wang et al., 2000). Our data suggest that eotaxin-1 may be more important for recruitment of eosinophils

Table 1
Summary of parameters of immunopathology following OVA immunization and challenge in CBA/Ca, C57BL/6 and BALB/c mice (this study and Tumes et al., 2007)

Immunopathology	Parameter	CBA/Ca	C57BL/6	BALB/c
Response to OVA challenge	Peripheral blood eosinophilia	+	+	+
	Total IgE	+++	++	+
	Airways pathology[a,b]	+	+++	+++
Eosinophil recruitment	Lung eosinophilia[c]	+	+++	++
	BAL IL-5[c]	++	++	+++
	Lung eotaxin-1	+	++	+++
	Eosinophil CD18 expression	+	++	+++
	Eosinophil L-selectin mRNA	+	+++	+
Eosinophil apoptosis	*Ex vivo* apoptosis[c]	++	+	+
	Bcl-2 expression	+	+	+++
	Bcl-x_L expression	+	+	+++

+ to +++ = increased compared to control or relative to other strains analysed.
[a] Goblet cell metaplasia, oedema, airways obstruction, BAL protein and EPO.
[b] Tumes et al. (2007).
[c] This study and Tumes et al. (2007).

by 72 h post-challenge, whereas other factors may be responsible for the smaller but significant increase in recruitment seen at 24 h. Importantly, eotaxin-1 levels were lowest in the CBA/Ca strain, in which eosinophil recruitment was also relatively modest.

The β1-integrin VLA-4 and the β2-integrins Mac1 and LFA-1 are expressed by eosinophils and facilitate the specific recruitment of these cells into the lungs through vascular (V) cell adhesion molecule (CAM-1) and intercellular (I) CAM-1, respectively (Walsh et al., 1991; Weller et al., 1991; Briscoe et al., 1992; Robinson et al., 1992; Schleimer et al., 1992; Nakajima et al., 1994; Pretolani et al., 1994; Das et al., 1995; Kitayama et al., 1997; Jia et al., 1999). We saw no variation in the expression of mRNA encoding VLA-4 in purified BAL eosinophils, or the protein CD11b (the α subunit of Mac1) at the cell surface. However CD18, which forms heterodimers with LFA-1, complement receptor (CR)4 and CD11d as well as Mac1, was expressed at higher levels on eosinophils from BALB/c and C57BL/6 mice than on cells from CBA/Ca animals. Lower expression of CD18 as part of LFA-1 and CD11d/CD18, receptors for the ICAM molecules, may result in less accumulation of eosinophils in the lungs of CBA/Ca mice, thus limiting the development of pathology in these animals. Eosinophils from the C57BL/6 strain express significantly higher levels of mRNA for the adhesion molecule L-selectin. This may explain in part the significantly greater presence of eosinophils in the lungs of C57BL/6 mice compared to the other two strains. This also highlights the likelihood that different mechanisms contribute to similar levels of pathology in the susceptible BALB/c and C57BL/6 strains.

The accumulation of eosinophils is not only dependent on recruitment but also the length of time these cells persist after entering tissues. Anti-Fas antibody treatment can induce apoptosis in human and mouse eosinophils and reduce eosinophilia in the lung following challenge (Matsumoto et al., 1995; Tsuyuki et al., 1995; Druilhe et al., 1996). However our data suggest that in this model eosinophils undergo apoptosis via the mitochondrial or intrinsic pathway, rather than the extrinsic pathway that is initiated through Fas or the TNF-related family of molecules. This is consistent with the observation that Fas-deficient (*lpr*) mice do not show a significant reduction in the number of BAL or airway tissue eosinophils following challenge (Duez et al., 2001).

Both wt and IL-5 Tg BALB/c mice had higher levels of BALF IL-5 than either of their CBA/Ca or C57BL/6 counterparts after heavy allergen challenge (Tumes et al., 2007) and a similar tendency was also seen with the light challenge conditions used in the present study. IL-5 has been shown to prolong eosinophil survival by modulating the expression of Bcl-2 and Bcl-x_L (Ochiai et al., 1997; Dibbert et al., 1998; Dewson et al., 1999; Villunger et al., 2000). BAL eosinophils recovered from BALB/c mice also had the highest levels of these anti-apoptotic proteins and this may contribute to the accumulation of eosinophils in this strain. In contrast, levels of Bcl-2, Bcl-x_L and IL-5 (this study and Tumes et al., 2007) in the lungs of the asthma-resistant CBA/Ca and susceptible C57BL/6 were generally lower than in the BALB/c mice. The pro-apoptotic proteins Bim and Bmf can regulate both spontaneous and drug-induced apoptosis of granulocytes (Villunger et al., 2003), but in this study no differences were seen between BAL cells from asthma-susceptible and asthma-resistant mice with respect to expression of either of these proteins.

While BALB/c and C57BL/6 mice develop similar airway pathology following immunization and challenge with OVA, it is likely that different mechanisms are contributing to the development of disease. Allergen-challenged BALB/c and C57BL/6 mice differ with respect to the relative importance of IL-4 and IL-5 to the development of pathology (Hsieh et al., 1995; Corry et al., 1996; Foster et al., 1996; Humbles et al., 2004; Lee et al., 2004), in the expression of TNF-α (Herz et al., 1998) and the localization of eosinophils in tissues (Takeda et al., 2001) and some of this may be due to a polymorphism in the IL-4 receptor α chain (Webb et al., 2004). In the current study we have further defined differences between these two commonly used mouse strains. Higher expression of IL-5 in the lungs may promote eosinophil survival in BALB/c mice via enhanced expression of anti-apoptotic proteins, whereas greater expression of L-selectin by eosinophils might facilitate recruitment in C57BL/6 mice. In contrast, CBA/Ca mice accumulate less eosinophils in the lungs after allergen challenge and this may be due to the combined effects of lower local production of eotaxin-1 and IL-5 and less expression of CD18 and L-selectin on eosinophils, than in one or both of the other strains.

In summary, disease pathogenesis in each of the strains examined in this study is likely to be subtly regulated by combinations of factors that influence the recruitment and survival of eosinophils. Our observations may translate into a better understanding of why some humans are more susceptible to the development of allergic asthma.

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