Deficiency of regulatory B cells increases allergic airway inflammation

S. K. Lundy1,2, A. A. Berlin2, T. F. Martens2 and N. W. Lukacs2

1 Department of Internal Medicine-Rheumatology, University of Michigan Medical School, Ann Arbor, MI 48109, USA
2 Department of Pathology, University of Michigan Medical School, Ann Arbor, MI 48109, USA, Fax: ++734 615 8166, e-mail: nlukacs@umich.edu

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Abstract. Objective: To investigate the effect of the X-linked immunodeficiency (Xid) B cell defect on the response to the cockroach allergen in mice. Methods: Two cockroach allergen immunization and challenge protocols were employed to sensitize CBA/J wild-type and CBA/CaHN-btk(-/-)xid/J (Xid) mice. Blood and tissue samples were collected 24 and 48 hrs after the last intratracheal antigen challenge and were analyzed for several parameters of allergic inflammation. Results: Nearly equivalent amounts of serum IgE were detected in Xid and CBA/J mice after short-term antigen challenge despite the B cell deficiency in Xid mice. A decreased concentration of IgE was detected in CBA/J mice after repeated allergen challenges but not in the Xid mice. Correlating with the discrepancy in serum IgE levels, higher levels of IL-13, IL-5, IL-10 and CCL5 were measured in whole lung homogenates from allergen-challenged Xid mice compared to CBA/J mice. In addition, draining lymph node cells from Xid mice expressed elevated levels of IL-4, IL-5, IL-10 and IFNγ mRNA compared to cells from CBA/J mice after in vitro culture with cockroach antigen. An increase in lung inflammation, interstitial eosinophilia and mucus production was also observed in allergen-challenged Xid mice. CD95L expression increased on B-1a cells following allergen challenge, which was accompanied by an increase in lung CD4+ Th cell apoptosis in wild-type CBA/J mice. In contrast, Xid mice did not have an increase in CD4+ T cell apoptosis following allergen challenge.

Conclusions: These data suggest a regulatory role for B-1a cells in reducing cytokine production, pulmonary inflammation, and CD4+ T cell survival during cockroach allergen-induced airway inflammation.

Key words: Allergy – B cells – T cells – Inflammation – Apoptosis

Introduction

Asthma is a chronic disease of the lung caused by exacerbated immune response toward environmental allergens. Much previous investigation has established that CD4+ T helper lymphocytes play a pivotal role in the establishment and maintenance of asthma. [1–3] These T helper cells produce Th2-associated cytokines including: IL-4, IL-5 and IL-13, which have pluripotent roles in promoting allergic reactions. [4, 5] In the setting of asthma, IL-4 stimulates differentiation of Th2 cells and proliferation of B cells, drives antigen-specific antibody production by B cells toward IgG1 and IgE isotypes, and inhibits production of the Th1-associated cytokine IL-12. [6–10] IgE binds to basophils and mast cells through surface Fce receptors, which when stimulated by antigen, cause the release of basophilic granules containing histamines and leukotrienes, that in turn stimulate the contraction of airway smooth muscle. [11–13] In asthma, IL-5 stimulates the production and maturation of eosinophils, which travel to the airways and release granular material, leading to mucus production and eventual airway remodeling in severe cases. [14] IL-13 shares some common activities with IL-4 through a common receptor, but has some non-redundant roles including being a major contributor to fibroblast stimulation and increased pulmonary fibrosis. [15–17] In addition to the Th2 cytokines, CD4+ T cells are a major source of chemokines that direct trafficking and activation of lymphocytes and...
leukocytes into inflamed airways. [5, 18] Thus, the CD4+ Th cell is an attractive target for understanding and regulating asthmatic responses.

A major, naturally occurring mechanism of CD4+ T cell regulation during inflammation is activation-induced cell death or apoptosis. Apoptosis is an important mechanism in the regulation of lung inflammation in asthma as indicated by an increase in chronicity when apoptosis is reduced. [19] Although histological analysis has not yet established an increase in total CD4+ T cell apoptosis in asthmatic patients, careful examination has revealed that T cells expressing IFN-γ are more apoptotic in asthmatics than in healthy subjects. [20] Although T cell apoptosis has been identified in asthmatics, the effector cells involved and the mechanisms employed to induce death are not completely understood.

It has recently been demonstrated that B cells, especially the CD5+/CD19+ B-1a cell subset, increase expression of surface Fas ligand (CD95L) in response to the Th2-inducing antigens of schistosome eggs. [21, 22] The B-1a cell subset is present in the pleural and peritoneal cavities, as well as the spleen but is not commonly found in lymph nodes. [23, 24] B-1a cells tend to recognize T-independent antigens such as lipids and carbohydrate moieties on glycoproteins and glycolipids, which are found in most common allergens. [25] Additionally, peritoneal B-1a cells have been shown to produce IL-10 upon antigenic stimulation. [26–28] These properties suggested a potential role for B-1a cells as a regulatory cell population at mucosal surfaces including the airways of the lung.

To test the hypothesis that B-1a cells contributed to the regulation of CD4+ T cells in asthma, cockroach allergen-induced (CRAg) models of asthma were performed in B-1a cell deficient, CBA/CaHN(btk-/-)Xid/J mice and compared to wild-type CBA/J mice. Increased serum IgE levels and airway inflammation were observed in allergen-challenged Xid mice. Lung cytokine levels were elevated in Xid mice and antigen-stimulated lymph node cells from Xid mice produced more Th1 and Th2 cytokines compared to wild-type mice. Decreased T cell death was also observed in allergen-challenged Xid mice. Thus, the B cell population missing from Xid mice appeared to play an important role in regulation of allergen-sensitized T cells and control of airway inflammation.

**Material and methods**

**Mice and Reagents**

Age-matched, female CBA/J and CBA/CaHN-btk(-/-)Xid/J (Xid) mice were purchased from Jackson Labs (Bar Harbor, ME) and housed under pathogen free conditions in the University Laboratory Animal Management facility at the University of Michigan Medical School. Mice were fed and drank ad libitum and were tested (negative) for the presence of many standard mouse pathogens. To ensure mutant status of mice, peritoneal lavage cells and collagenase dispersed lung cells were stained with anti-mouse CD19-FITC and anti-mouse CD5-PE antibodies (Pharmingen, San Diego, CA) and analyzed by two-color flow cytometry. All Xid mice completely lacked CD5+/CD19+ double positive B cells and had an approximately 50% reduction in CD5-/CD19+ B cells. [29] There were no detectable differences in growth, behavior or health between the two groups of mice throughout the course of the experiments. Skin test-grade cockroach antigen (CRAg) extracts were purchased from Hollister Stier (Spokane, WA) and tested negative for bacterial lipopolysaccharides by limulus amoebocyte assay. Antibodies for flow cytometry and other reagents were purchased from BD/Pharmingen (San Diego, CA) and Sigma/Aldrich (St. Louis, MO).

**Cockroach Allergen Sensitization and Challenge**

Mice (8–10 weeks old) were injected i.p and s.c. with 0.1 ml of cockroach allergen (CRAg, 20,000 protein nitrogen units/ml) emulsified 1:1 in Incomplete Freund’s adjuvant (Sigma) on day 0. In the standard challenge model, mice received an intranasal challenge with 15 µl of undiluted CRAg on day 14, followed by intratracheal injections of 40 µl CRAg on days 21 and 23. In the chronic challenge model, mice received intranasal challenges on days 14, 18, 22, 26 and 30, followed by intratracheal challenges on days 34 and 38. In both models, mice were tested for methacholine-elicted airway hyperreactivity (see description below) at 16–20 hours after the final i.t. challenge, unless otherwise indicated, then immediately sacrificed for immunological and histological analysis.

**Serum IgE Detection**

Blood was obtained by retro-orbital eye bleed of anesthetized mice at the time of sacrifice and allowed to clot at 4°C for 1 hr prior to removal of serum. ELISA plates were coated with polyclonal anti-Ig (BD Pharmin-gen), washed and incubated with serum for 1 hr. Monoclonal anti-IgE-biotin conjugated antibody and streptavidin-horseradish peroxidase were used for detection of serum IgE. Data represent the combined results of six experiments.

**Measurement of Lung Cytokine Levels by ELISA**

The left lobe was removed from chronically challenged CBA/J and Xid mice 16–20 hours after the final i.t. challenge and snap frozen. Lung tissue was homogenized in 1 ml PBS buffer containing Triton X-100 and protease inhibitors, and debris removed by high speed centrifugation. Samples were loaded onto ELISA plates precoated with anti-murine cytokine/chemokine antibodies (R&D Systems, Minneapolis, MN). Sandwich ELISA was performed following manufacturer’s instructions and recombinant cytokines were used to generate a standard curve for each assay. Data are from a representative experiment of three performed.

**Real-time PCR for Cytokine Expression in Cultured LN Cells**

Mediastinal lymph nodes were removed from chronically challenged CBA/J and Xid mice at 16–20 hours post-i.t. challenge. Cells were dispersed through a mesh screen, counted and plated in triplicate (5x10^6 cells/ml) in the presence or absence of 200 PNU/ml of CRAg. Cells were harvested 16 hrs after initiation of culture, placed in Trizol reagent (Invitrogen, Carlsbad, CA), and snap frozen. Total RNA was purified following manufacturers instructions and mRNA was reverse transcribed using oligo-dT and MMLV-RT from Invitrogen. Real-time PCR was performed using an ABI Prism 7700 Sequence Detector and reagents from PE Biosystems (San Diego, CA). Individual samples were normalized to an internal GAPDH amplification control, then mRNA expression was compared between media control and CRAg-stimulated samples for each lymph node cell population. Data are expressed as the mean fold increase in mRNA expression ± standard error for the triplicate samples of a representative experiment of the three performed.

**Histological Analysis of Peribronchiolar Eosinophilia**

Right lung lobes were removed from chronically challenged CBA/J and Xid mice 16–20 hrs after the final i.t. challenge and inflated with 10% neutral buffered formalin. Lungs were embedded in paraffin, sectioned