consequences of adenosine deaminase deficiency in mice are associated with defects in alveogenesis, pulmonary inflammation, and airway obstruction. J Exp Med 2000; 192:159–170

Overlap Between Death Receptor and Non-Receptor-Mediated Mechanisms During Apoptosis in Human Eosinophils*

Sultan Niazi, MD; Noreen M. Robertson, DMD; Ashish Agrawal, MD; Annette T. Hastie, PhD; Stephen P. Peters, MD, PhD; FCCP; and James Zangrilli, MD, FCCP

(CHEST 2003; 123:345S)

Abbreviations: Dex = dexamethasone; IL = interleukin

Eosinophils undergo spontaneous apoptosis in the absence of specific cytokines, which is further accelerated by Fas engagement and glucocorticoids. We have previously shown that interleukin (IL)-5 profoundly affects the processing of caspase 3 in eosinophils, a downstream executor of apoptosis, but the level of inhibition is unclear.1 We hypothesized that IL-5 would affect the kinetics of receptor-mediated apoptosis vs non-receptor-mediated apoptosis differently and that this would be reflected by differential activation of elements presumed to be unique to these pathways.

MATERIALS and METHODS

Human peripheral blood eosinophils were cultured in media alone (control), IL-5 (1 ng/mL), dexamethasone (Dex; 1 μM/mL), or agonist anti-Fas antibody (500 ng/mL). Viability by trypan, and the processing of caspases 8, 9, and 9 apoptosis regulatory protein Bid by immunoblot assay were determined at 8, 24, and 48 h. Bid also was examined in fixed eosinophils by immunocytochemistry.

RESULTS

Anti-Fas and Dex accelerated cell death compared to media alone. IL-5 blocked Dex-induced cell death and slowed cell death induced by anti-Fas. Caspase 8, 9, and Bid processing occurred during both anti-Fas and Dex treatment. Bid was processed to a similar extent during both anti-Fas and Dex stimulation. Furthermore, Dex treatment caused an early shift in cellular Bid staining from its baseline diffuse cytoplasmic pattern to an increasingly punctate one.

CONCLUSION

Caspases 8, 9, and Bid were processed efficiently in the absence of obvious activation of the Fas receptor, suggesting alternative mechanisms for caspase 8 activation. IL-5 had the ability to modulate the caspase activation at several levels.

REFERENCE


Isolation and Characterization of Hemopoietic Cells From Lungs of Allergic Mice*

Maria Izig C. Gaspar Elsas, MD, PhD; Elisabeth S. Maximiano, PhD; Danielle Joseph; Adriana Bonomo, MD, PhD; Bernardo Boris Vargaftig, MD, PhD; Pedro Xavier Elsas, MD, PhD

We developed a procedure for the isolation of hemopoietic cells from murine lung. Ovalbumin sensitization and challenge increased the numbers of functionally intact hemopoietic progenitors recovered from digested lung fragments by 50-fold to 120-fold, relative to naive controls. Eosinophil precursors, which are absent in the naive mouse lung, accumulated in the lungs of sensitized/challenged mice. Progenitors in allergic BALB/c mice were recoverable from lung parenchyma, not blood or airways, and were exclusively CD34+. Precursors isolated from allergic lung, unlike those from bone marrow, were inhibited by dexamethasone and were stimulated by prostaglandin D2. This directly demonstrates that sensitized/challenged lungs accumulate hemopoietic progenitors and precursors, distinct from those in bone marrow.

(CHEST 2003; 123:3455S–3488S)
Abbreviations: EPO = eosinophil peroxidase; FCS = fetal calf serum; GM-CSF = granulocyte-macrophage colony-stimulating factor; IL = interleukin; IMDM = Iscove modified Dulbecco medium; OVA = ovalbumin

In humans, the accumulation of CD34+/interleukin (IL)-5 receptor-α messenger RNA+ cells, which presumably include eosinophil progenitors, has been described in the allergen-challenged lung. However, direct evidence for the presence of functionally intact hemopoietic progenitors in the lungs has not yet been reported because this involves procedures and assays that are difficult to carry out with human lungs. Here we describe a procedure for the quantitative recovery of fully functional hemopoietic cells, which accumulate in allergen-challenged mice, from murine lungs.

MATERIALS AND METHODS

Animals and Animal Procedures

BALB/c mice were immunized following either of the following protocols: (1) sensitization by two subcutaneous 0.4-mL injections of 100 μg ovalbumin (OVA) mixed with 1.6 mg Al(OH)₃ (alum) in 0.9% NaCl (saline solution), at 7-day intervals, followed by intranasal challenge with 10 μg OVA in 50 μL saline solution, 1 week after the second injection; and (2) sensitization at day 0 by one intraperitoneal 0.2-mL injection of 10 μg OVA mixed with alum in saline solution, followed by intranasal challenge at 24-h intervals with 20 μg OVA in a 50-μL saline solution from day 10 to day 13. Controls were either naive or sensitized mice that had been challenged with saline solution. Animals were killed 24 h after the last challenge. Mice that were anesthetized with thiopental were exsanguinated from the abdominal vena cava. The heparinized blood was used for hemopoietic cell purification. After opening the thoracic cage, an incision was made in the right atrium, and the remaining blood was washed out of the lungs by pumping sterile saline solution through the left atrium. The effectiveness of this procedure in clearing the pulmonary microcirculation was confirmed by histologic examination of anti-CD34-stained lung sections (Tissue-Tek: Elkhart, IN). The lungs were excised and minced in Iscove modified Dulbecco medium (IMDM) in a Petri dish. BAL fluid, blood, and/or spleen cells, in addition to lung tissue, were harvested from naive or sensitized mice.

*From the Departamento Pediatria (Dr. Elsas), Institute Fernandes Figueira, FIOCRUZ, Rio de Janeiro, Brazil; Department of Immunology (Dr. Maximiano, Bonomo, and Elsas), Instituto Microbiologia Professor Paulo de Góes, UFRJ, Rio de Janeiro, Brazil; and Unité de Pharmacologie Cellulaire (Ms. Joseph and Dr. Vargaftig), Unité Associée Institut National de la Santé et de la Recherche Médicale-Institut Pasteur U485, Paris, France. This research was supported by Institut National de la Santé et de la Recherche Médicale (INSERM)-Leprosy Care Outpatient Clinic of the Oswaldo Cruz Foundation (FIOCRUZ), Fundação de Estudos e Pesquisas, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Conselho Nacional do Desenvolvimento Científico e Tecnológico, and the Institut Pasteur.

Correspondence to: P. Xavier Elsas, MD, PhD, Associate Professor, Department of Immunology, Instituto de Microbiologia Prof. Paulo de Góes, Universidade Federal do Rio de Janeiro, CCS, Bloco I, 2º andar, sala 066, 21941-590, Rio de Janeiro, Brazil; e-mail: pxelsas@yahoo.com.br.

Isolation and Quantification of Hemopoietic Cells

Turk solution was used for nuclear cell counts; trypan blue was used for the assessment of viability and staining for eosinophil peroxidase (EPO) for the identification of mature and immature eosinophils. Minced lungs were incubated for 20 min at 37°C, with a mixture of 95% air/5% CO₂ in 10 mL IMDM containing 24 mg collagenase and 0.125 mg DNASE 1, before the addition of fetal calf serum (FCS; 20% final) to stop digestion. Lung cell suspensions were obtained by passing fragments through a cell strainer (Falcon 2930) [100 μm, nylon], washed twice, counted, and separated on discontinuous gradients (Percoll; Amersham Bioscience; Little Chalfont, Buckinghamshire, UK) [75%, 60%, and 40% Percoll in IMDM] for 20 min at 100g, at 20°C, with 2 x 10⁶ cells per gradient. The low-density mononuclear cells at the 40 to 60% Percoll interface (layer 2) were harvested, washed twice in RPMI medium/FCS, counted, and used for EPO staining and hemopoietic assays. Where indicated, they were stained with rat antimouse CD34 antibody and goat anti-rat IgG fluorescein isothiocyanate conjugate, and were analyzed by flow cytometry (FACScalibur; Becton Dickinson Biosciences; Franklin Lakes, NJ) or were further incubated with a fluorescein isothiocyanate–conjugated (Microbeads; Miltenyi Biotech GmbH; Cologne, Germany) for 15 min and separated (Midimacs columns; Miltenyi Biotec). For bone-marrow, BAL fluid, blood, and spleen cells, triplicate 1-mL semi-solid cultures were plated in methylcellulose in 35-mm culture dishes from 2 x 10⁶ cells, in IMDM with 20% FCS, granulocyte-macrophage colony-stimulating factor (GM-CSF) [2 ng/mL], IL-3 [2 ng/mL], and IL-5 [1 ng/mL]. Colonies were scored at days 7 and 10. Scoring after day 7 did not result in better colony detection, but colony morphology was less preserved. For comparison with lung-derived colonies (see below), only counts at day 10 are presented. Unlike bone marrow, colony formation by lung mononuclear cells required the presence of methylcellulose and higher cell densities. Cultures were routinely established with 4 x 10⁶ cells in a 1-mL volume, in triplicate. Colonies derived from lung cells grew more slowly than those from bone marrow, but their morphology resembled eosinophil colonies derived from bone marrow. As a rule, counting at day 10 yielded higher counts of lung-derived colonies than at day 7, due to improved colony detection with well-preserved colony morphology. Results always refer to counts at day 10. For typing, individual colonies were aspirated with a micropipette under the inverted microscope, were transferred to microslide slides, and were stained (Panoptic stain or EPO). Results are calculated as the mean ± SEM of progenitor numbers per animal, based on the number of colonies formed by a standard inoculum (lung, 4 x 10⁶ cells; BAL and spleen, 2 x 10⁶ cells) and on the number of mononuclear cells recovered per animal in each cell source. For blood, the results are presented per milliliter of blood. For all sources of hemopoietic cells, liquid cultures (10⁶ cells in a 1-mL volume, in a 24-well cluster) were seeded in RPMI medium/10% FCS, 2 mmol/L L-glutamine, and penicillin-streptomycin at 37°C, in an atmosphere of 5% CO₂/95% air, at least in triplicate, in the presence of GM-CSF (2 ng/mL), IL-3 (2 ng/mL), and IL-5 (1 ng/mL). The frequency of EPO+ cells was determined at day 7.

RESULTS AND DISCUSSION

Significantly higher numbers of low-density mononuclear cells were recovered from sensitized/challenged mice than from naive controls, with an approximate 10-fold increase in mice that were challenged repeatedly with OVA. Very few or no colony-forming cells were present among lung mononuclear cells from naive mice. Sensitization by itself did not significantly increase pro-
genitor numbers relative to naive controls. Progenitor numbers were significantly increased in mice sensitized following protocol 1 and challenged with OVA, relative to both naive controls ($p = 0.003$) and sensitized controls ($p = 0.047$). Repeated challenges of sensitized mice (protocol 2) did not significantly increase progenitor numbers relative to protocol 1. When lung mononuclear cells were separated into CD34$^{+}$ and CD34$^{-}$ subpopulations, colonies were formed only from CD34$^{+}$ cells. The frequency of colony-forming cells in the low-density mononuclear fraction from sensitized/challenged lung cells was roughly 1 in every 4,000 cells. This represents an approximate 100-fold enrichment over naive cells from BALB/c mouse lung. For comparison, myeloid progenitors responsive to GM-CSF represent about 1 in every 2,000 bone marrow cells for both naive and sensitized BALB/c mice in our routine culture conditions. Hence, hemopoietic progenitors were approximately one half as frequent in allergic lung as in allergic bone marrow. Eighty-four percent of all lung-derived colonies were EPO$^{+}$, indicating a strong bias toward eosinopoiesis. No significant response to IL-5 was observed in liquid cultures from naive or from sensitized BALB/c mice in the absence of OVA challenge. In contrast, a vigorous response was seen in cultures from challenged, sensitized mice that was comparable in magnitude to that elicited in challenged, sensitized bone marrow. The findings, taken together, indicate that hemopoietic progenitors and precursors selectively accumulate in the murine lung after allergen sensitization and challenge. BAL fluid from sensitized/challenged mice contained very small numbers of colony-forming cells, while large numbers of progenitors could be recovered from digested lung fragments of the same animals. All colonies from BAL were EPO$^{+}$. This shows that progenitor accumulation in the lung cannot be estimated from the numbers of progenitors in BAL fluid. On the other hand, blood and spleen did contain colony-forming cells. The total number of colony-forming cells in the circulation (assuming a total blood volume of 2 mL for the mouse) amounted to two thirds of the total number of progenitors isolated from lung.

Hemopoietic cells isolated from allergic BALB/c mice lungs differed from those in the bone marrow of the same animals in terms of their sensitivity to pharmacologic...
modulators. As shown in Figure 1, dexamethasone enhanced the responses of eosinophil precursors from bone marrow to IL-5 but inhibited the responses of eosinophil precursors from the lung under the same conditions. Because, in current models, the suppression of tissue eosinophilia is thought to underlie the therapeutic effects of steroids, hemopoietic cells accumulating in the lungs, unlike those in bone marrow, respond to dexamethasone as predicted from these models. This highlights potentially important differences between these hemopoietic populations.

REFERENCES


Contribution of the Distal Lung to the Pathologic and Physiologic Changes in Asthma*

Potential Therapeutic Target

Roger S. Mitchell Lecture

Meri K. Tulic, PhD; and Qutayba Hamid, MD

Pathologic and physiologic evidence has emerged in the last few years suggesting that the airway inflammation and remodeling that characterize asthma occur not only in the central airways but extend to the distal lung and the lung parenchyma. The distal airways are capable of producing T helper (Th) type 2 cytokines and chemokines, and, more recently, they have been recognized as a predominant site of airflow obstruction in asthmatic patients. In the lung parenchyma, a similar Th2 cytokine profile and infiltration of inflammatory cells also has been reported. The inflammation at this distal site has been described as being more severe when compared to the large amount of airway inflammation, and evidence of remodeling in the lung periphery is emerging. The recognition of asthma as a disease of the entire respiratory tract has an important clinical significance highlighting the need to also consider the distal lung as a target in any therapeutic strategy for effective treatment of this disease.

(CHEST 2003; 123:348S–355S)

Key words: allergic inflammation; asthma; distal airways; small airways

Abbreviations: BDP = beclomethasone dipropionate; CFC = chlorofluorocarbon; GR = glucocorticoid receptor; HFA = hydrofluoroalkane; IL = interleukin; MCh = methacholine; MDI = metered-dose inhalers; NA = nocturnal asthma; NNA = nonnocturnal asthma

Asthma is a chronic inflammatory disease that is characterized by episodic airway obstruction and increased bronchial responsiveness. The major pathologic and structural features of asthma include epithelial shedding, airway smooth muscle hypertrophy and hyperplasia, mucous gland hyperplasia, subepithelial fibrosis, and infiltration of the bronchial wall with inflammatory cells. The concept that inflammation is a major component of asthmatic pathology was established >100 years ago. These studies have used autopsy specimens to study the macroscopic, morphologic, and histologic changes within the large airways. That the distal airways and the lung parenchyma play a role in asthma has been suggested by experiments in which the physiologic behavior of the lung has been investigated. These early studies conducted from the Meakins-Christie laboratories1–3 focused attention on the role of the distal airways in asthma; however, investigation in this area lagged because of the difficulties in examining these peripheral structures directly. Since then, the development of new techniques with which to measure lung physiology has demonstrated the distal site to be recognized as a predominant site of airflow obstruction in asthmatic patients.3–6

The introduction of fiberoptic bronchoscopy techniques has enabled us to obtain small human endobronchial biopsy specimens from the large airways of asthmatic patients and, together with the recent applications of molecular pathology techniques, to advance our understanding of the pathogenesis of bronchial asthma. Studies utilizing these approaches in surgically resected lung tissue,5,6 postmortem lung specimens,9–12 and transbronchial biopsy specimens11,14 have demonstrated that similar but more severe inflammatory and structural changes also occur in the distal lung and lung parenchyma of asthmatic patients. It is now accepted that in asthmatic patients, the

*From the Meakins-Christie Laboratories, McGill University, Montreal, PQ, Canada. The authors would like to acknowledge MRC Canada, GlaxoSmithKline and 3M Pharmaceuticals for their support. Dr. Tulic is a Ludwig-Engel Post-Doctoral Fellow, and Dr. Hamid is a recipient of the Senior Fonds de la Recherche en Santé du Québec Chercheur-Boursier Award. Reproduction of this article is prohibited without written permission from the American College of Chest Physicians (e-mail: permissions@chestnet.org).

Correspondence to: Qutayba Hamid, MD, PhD, Professor of Medicine, Meakins-Christie Laboratories, McGill University, 3626 St. Urbain St, Montreal, PQ, Canada H2X 2P2; e-mail: qutayba.hamid@mcgill.ca

Thomas L. Petty 45th Annual Aspen Lung Conference: Asthma in the New Millennium