Airway epithelial Fas ligand expression: potential role in modulating bronchial inflammation

BERNADETTE R. GOCHUICO, EDITH M. HESSEL, JORIS J. DE BIE, ANTOON J. M. VAN OOSTERHOUT, WILLIAM W. CRUIKSHANK, and ALAN FINE

1The Pulmonary Center, Boston University School of Medicine, Boston, Massachusetts 02118; and 2Department of Pharmacology and Pathophysiology, Utrecht Institute for Pharmaceutical Sciences, 3508 TB Utrecht, The Netherlands

Fas activation is initiated by binding to the Fas ligand (FasL), a type II transmembrane protein belonging to the tumor necrosis factor family of ligands (15, 18, 26). FasL is expressed in various tissues including bronchial epithelium, the spleen, thymus, lymph nodes, and lung (8, 9, 26). Within lymphoid tissues, FasL is expressed predominantly in activated T cells, playing a role in cytotoxic activity (11). In contrast, the cell types expressing FasL in nonlymphoid tissues have not been completely identified. In the eye, FasL expression has been demonstrated in the corneal epithelium and certain cell populations of the anterior and posterior chambers of the eye, whereas in the testis, FasL expression has been uniquely localized to Sertoli cells (8, 9). Experimental data suggest that expression of FasL at these sites protects against tissue injury by inducing apoptosis in infiltrating immune cells during immunologic reactions and infection (1, 8, 9).

The airways of the lung, through their continuous exposure to airborne antigens, would also benefit from mechanisms that protect against immune reactivity. Although activation-induced cell death of lymphocytes within the lung may participate in preventing inflammation induced by chronic antigenic exposure (13), alternative protective mechanisms, perhaps involving the epithelium, are possible. In this regard, the experimental removal of the airway epithelium in guinea pigs results in infiltration of the airway wall by eosinophils; subsequent restitution of the epithelium is associated with eosinophilic apoptosis (6). It is notable, therefore, that in this paper we report the localized expression of FasL to airway epithelium. The gld mice that express mutated FasL were noted to develop peribronchial accumulation of mononuclear cells. Moreover, during chronic allergen-induced airway inflammation, downregulation of FasL expression occurred. We believe these novel findings may have broad implications for understanding the regulation of immune cell infiltration of the airway and the pathogenesis of diseases such as asthma that are characterized by persistent inflammation.

METHODS

Ovalbumin sensitization and challenge. The methods are essentially those of Hessel et al. (12). Experimental procedures were performed according to institutional guidelines as outlined by Boston University and the Utrecht Institute for Pharmaceutical Sciences.
Processing of lung tissue. Mice were killed by cervical dislocation followed by intra-abdominal aortic exsanguination. The lungs were fixed by intratracheal inflation with freshly prepared 4% paraformaldehyde in phosphate-buffered saline (PBS). The lung tissue was then fixed in 4% paraformaldehyde in PBS at 4°C overnight, washed in 0.85% NaCl, dehydrated in graded ethanol solutions, immersed in xylene, and embedded in filtered paraffin. The tissue was sectioned at 6 µm and placed on Vectabond (Vector Laboratories, Burlingame, CA)-treated glass slides.

Generation of digoxigenin-labeled FasL riboprobe for in situ hybridization. Probes were synthesized with a 290-base pair (bp) murine FasL cDNA sequence corresponding to a unique 3' region. This sequence was subcloned into pBluescript, and linearized plasmid was incubated with either T7 (2 U/µl) or T3 (5 U/µl) RNA polymerase (Boehringer Mannheim, Indianapolis, IN), polymerase buffer, digoxigenin (Dig)-labeled UTP, and other precursor nucleotides at 37°C for 2 h. Unincorporated nucleotides were removed after ethanol precipitation with a glycogen carrier. With the utilization of a standard curve obtained by probing known concentrations of a Dig substrate, antisense and sense probe concentrations were estimated by dot-blot analysis.

Nonisotopic in situ hybridization. The methods are essentially those of Panoskaltsis-Mortari and Bucy (23). Tissue sections derived from adult wild-type C57BL/6 mice (19–21 g; Charles River Laboratories, Wilmington, MA) were hybridized with either antisense or sense Dig-labeled FasL riboprobes to analyze FasL expression within the normal murine lung. Similarly, sections derived from paired ovalbumin (OA) model mice were hybridized with FasL riboprobes to compare FasL expression within this murine model of asthma.

Immunohistochemistry. Tissue sections derived from adult wild-type C57BL/6 mice (19–21 g; Charles River Laboratories) were employed to analyze FasL expression within the normal murine lung. Processing was performed according to instructions outlined in the Vectastain Elite ABC Kit (Vector Laboratories). A primary mouse anti-murine FasL monoclonal antibody [immunoglobulin (Ig) G2b (17) and a secondary biotinylated polyclonal horse anti-mouse IgG conjugate were sequentially employed on experimental sections. Control tissue sections derived from paired ovalbumin (OA) model mice were hybridized with FasL riboprobes to compare FasL expression within this murine model of asthma.

Results

FasL expression in the normal lung. To localize FasL expression within tissue sections derived from the normal murine lung, we performed nonisotopic in situ hybridization and immunohistochemistry. In situ hybridization with a Dig-labeled FasL cRNA probe revealed an intracytoplasmic colorimetric signal diffusely within tracheal and bronchial Clara cells (nonciliated, cuboidal airway epithelial cells; Fig 1, a and b) (19). A less prominent nonisotopic signal was also noted in some ciliated and squamous cells of the upper airway. Staining was also seen in rare, scattered alveolar type II cells but not in fibroblasts, macrophages, or endothelial cells (data not shown). Hybridization with a sense probe did not generate a signal (Fig 1, c and d).

Consistent with FasL mRNA expression in the airway epithelium, FasL protein was localized to the cell membrane and cytoplasm of surface epithelial cells throughout the trachea, bronchi, and bronchioles (Fig. 2). Staining was present in most Clara cells and some ciliated and squamous epithelial cells. Immunolocalization of FasL protein was also detected in some alveolar type II cells (data not shown). Lung sections incubated with only the secondary antibody did not contain immunostaining (data not shown).

Histological characteristics of gld mutant mouse lung. To elucidate the possible role of airway epithelial FasL, we performed hematoxylin and eosin staining of lung sections derived from adult gld mutant mice. These mice contain a loss-of-function point mutation in the COOH-terminal region of the FasL protein (18). Examination of the trachea and bronchovascular bundles in this mutant mouse revealed accumulation of mononuclear inflammatory cells within the submucosa and perivascular space (Fig. 3a). Several features of the mononuclear cell infiltration indicated that these changes did not represent accentuated bronchus-associated lymphoid tissue (3). First, on serial sectioning, no discrete border was evident. Second, the pattern of infiltration, particularly in the upper airway, was diffuse rather than the focal nodular lymphoid aggregation that typifies bronchus-associated lymphoid tissue. Notably, this mononuclear cell infiltration was not seen in an age-matched control animal with the same background genotype (Fig 3b). These findings are compatible with previous reports demonstrating increased numbers of activated CD4+ and CD8+ lymphocytes and double-negative T cells in lungs derived from adult gld mice (14). Inspection of the lung parenchyma revealed grossly normal alveolar structures.

Expression and release of FasL in a murine model of asthma. To examine the expression of airway epithelial cell FasL during active inflammatory processes of the airway, we employed the OA model of allergic inflammation (2, 12). OA-sensitized mice develop airway hyperresponsiveness and prominent inflammatory cellular infiltration 24 h after OA challenge, whereas OA-sensitized mice exposed to saline (control) do not develop an inflammatory reaction. To determine whether FasL expression was altered in this model, in situ hybridization and immunohistochemistry were performed on sections derived from multiple, paired animals sensitized to OA and challenged with either aerosolized OA (n = 5) or saline (n = 5). As shown in Fig. 4, a marked reduction in airway epithelial staining for FasL mRNA and protein was detected in OA-
challenged animals (a and c) compared with paired saline control animals (b and d). These findings were observed most prominently in Clara cells throughout the proximal and distal epithelia, which remained intact under both experimental conditions. Notably, FasL mRNA and protein were expressed in some peribronchial mononuclear inflammatory cells within tissue derived from OA-challenged mice (Fig. 4, a and c).

DISCUSSION

Fas-mediated apoptosis is thought to be involved in T-cell deletion and cytotoxicity, modulation of the inflammatory response, maintenance of the immune privileged status of the eye and testis, tumor evasion from host immune response, and regulation of turnover in certain epithelial cell populations (1, 7–10, 21, 25, 26). The biological function of the Fas signaling system is consistent with the phenotypes exhibited by the lpr and
gld mouse strains; these mice develop autoimmunity and generalized lymphoproliferation (4). Chromosomal mutations in these animals result in loss of expression of Fas and dysfunctional FasL expression, respectively (4, 18, 29). Similar features of lpr and gld mice have been observed in humans with various disorders. The pathophysiology of these diseases is believed to be related to alteration of Fas-mediated apoptosis. For example, upregulation of Fas has been demonstrated in peripheral lymphocytes isolated from patients with systemic lupus erythematosus (20). Also, loss-of-function Fas gene mutations have been detected in T cells from patients with the Canale-Smith syndrome, a disorder with lymphoproliferative features (24).

In addition to immune cell aggregation in lymphoid tissues, lpr and gld mutant mice accumulate lymphocytes in epithelial tissues such as the small intestine, female genital tract, and lung. These sites are notable for containing mucosal surfaces that are chronically exposed to environmental antigens. In the gld lung, T cells accumulate and express markers of chronic activation, including CD69 (14). These aggregations of peribronchial, submucosal lymphocytic cells in the gld mouse coupled with our FasL expression data suggest that Clara cell–derived FasL contributes to modulation of immune activity in the airway. This hypothesis is in agreement with the postulated immunoregulatory role of FasL in other epithelial tissues such as the eye and testis and in epithelial malignancies such as bronchogenic lung cancer and melanoma (1, 8–10, 21).

Nonciliated, cuboidal bronchiolar epithelial cells (Clara cells) have diverse functions in homeostasis and disease (19). These secretory, surface epithelial cells are a heterogeneous population that varies in morphological features, protein expression, and susceptibility to aerosolized toxin injury. Clara cells synthesize multiple substances such as Clara cell 10-kDa protein and/or uteroglobin, surfactant proteins, Clara cell tryptase, and leukocyte protease inhibitor. These epithelial cells are believed to function predominantly in xenobiotic metabolism by cytochrome P-450 oxidase activity, repopulation of airway epithelium, and tumor generation. Our results, therefore, suggest a novel role for Clara cells in controlling airway inflammation via FasL expression.

Consistent with this possible immunomodulatory role of Clara cell–derived FasL, we found a marked reduction in airway epithelial cell FasL mRNA and protein expression during allergen-induced airway inflammation. The submucosal immune cell accumulation occurred despite FasL expression by some of the mononuclear cells located within the inflammatory aggregates. In previous work, mice sensitized and challenged with OA developed airway hyperreactivity...
Bronchoalveolar lavage fluid and peribronchial tissue derived from these experimental mice contained increased numbers of neutrophils, eosinophils, and lymphocytes 24 h after OA challenge. Notably, these immune cells are Fas bearing (11, 16, 27).

In conclusion, our results suggest that the downregulation of expression of airway epithelial FasL may be a

Fig. 4. FasL expression after ovalbumin challenge. In situ hybridization and immunohistochemistry were performed on lung sections derived from paired ovalbumin (a and c)- or saline (b and d)-challenged mice. Hybridization with antisense digoxigenin RNA probe generated red-purple color signal (>180; a and b); hybridization with sense probe at matched concentrations did not generate signal (data not shown). Representative fields from cross-sectioned and longitudinally sectioned distal airways are displayed. Loss of epithelial Fas ligand is noted after ovalbumin (a) vs. saline (b) challenge. Large arrow, peribronchial inflammation; small arrowheads, some mononuclear cells containing signal. c and d: tissue sections that were sequentially incubated with matched concentrations of primary polyclonal anti-FasL antibody, secondary biotinylated anti-rabbit antibody, avidin-biotin-horseradish peroxidase complex, and color substrate (>100). Peroxidase-generated signal is indicated by brown staining. Loss of epithelial Fas ligand immunoreactivity is displayed after ovalbumin (c) vs. saline challenge (d). Cell surface-stained peribronchial inflammatory cells are indicated by small arrows. Incubation with only the secondary antibody did not produce color signal (data not shown). Sections were counterstained with methylene green. Photomicrograph exposures were identical.

and inflammation in a stereotypic manner (2, 12). Bronchoalveolar lavage fluid and peribronchial tissue derived from these experimental mice contained increased numbers of neutrophils, eosinophils, and lymphocytes 24 h after OA challenge. Notably, these immune cells are Fas bearing (11, 16, 27).
contributing factor to the evolution of persistent airway inflammation. During the chronic antigen exposure that characterizes lung homeostasis, we speculate that FasL derived from the airway epithelium functions to prevent the accumulation of immune effector cells. The establishment of an inflammatory state, however, likely involves alterations in the balance of Fasl availability relative to the pace of inflammatory cell accumulation. The resultant airway pathology of the gld mouse may be one such example in which this balance is disrupted. Another example may be the OA-challenged mouse. In this model, diminished FasL expression in the airway epithelium along with the concomitant inflammatory response 24 h after OA challenge may exceed the capacity of Fas-dependent mechanisms to modulate inflammatory cell accumulation. Overall, future studies are needed to further clarify relationships between Fasl expression in the lung and the development and pathogenesis of airway inflammation.

This work was supported by a Grant-In-Aid from the American Heart Association, National Heart, Lung, and Blood Institute Grant HL-56386, and Asthma, Allergic, and Immunologic Diseases Cooperative Research Center Grant AI-41994.

Address for reprint requests: B. R. Gochuico, The Pulmonary Center, Boston Univ. School of Medicine, 80 East Concord St., R304, Boston, MA 02118.

Received 30june 1997; accepted in final form 28December 1997.

REFERENCES

7. Enari, M., R. V. Talanian, W. W. Wong, and S. Nagata. FasL derived from the airway epithelium functions to modulate inflammatory cell accumulation. Overall, future studies are needed to further clarify relationships between Fasl expression in the lung and the development and pathogenesis of airway inflammation.
11. Hanabuchi, S., M. Koyanagi, A. Kawasaki, N. Shinohara, A. Matsuzawa, Y. Nishimura, Y. Kobayashi, S. Yonehara, H. Yagita, and K. Okumura. Lung inflammation and the development and inflammatory cell accumulation. Overall, future studies are needed to further clarify relationships between Fasl expression in the lung and the development and pathogenesis of airway inflammation.