MORAXELLA CATARRHALIS-SPECIFIC TH1 CELLS IN BAL FLUIDS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE PATIENTS

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In chronic obstructive pulmonary disease (COPD) patients' airway mucosa is infiltrated by macrophages and T lymphocytes, potentially reactive to pathogens. We studied the antigen-specificity and the effector functions of *in vivo* activated T lymphocytes isolated from BAL (Bronchoalveolar lavage) of 5 *Moraxella catarrhalis* (Mc)-infected and 5 Mc-non-infected COPD patients. Mc-specific T cells were detected only in BAL or peripheral blood of *Moraxella catarrhalis*-infected patients. The majority of BAL Mc-specific T cells expressed the T helper type 1 (Th1) cytokine profile with high cytotoxic and pro-apoptotic activity. Upon antigen stimulation, all Mc-specific T clones were able to help the immunoglobulin production by autologous B cells and the MMP (Matrix MetalloProteinase)-12 activity by monocytes. Our results suggest a role for Th1-driven response to *Moraxella catarrhalis* in the genesis of COPD.

Chronic Obstructive Pulmonary Disease (COPD) is a serious global epidemic disease predicted to become the third most common cause of death by the year 2020. The major risk factor is cigarette smoking, but air pollution and other irritatants in industrialized countries can contribute to the development of the disease (1). Emphysema is a major pathology of COPD (2). The clinical syndrome of COPD involves both pulmonary manifestations, including airflow obstruction, small airway inflammation (bronchiolitis), and lung parenchyma (alveolar) destruction (3), and extrapulmonary manifestations (muscle wasting, osteoporosis, anemia) (4).

COPD is characterized by intermittent exacerbations and worsening of symptoms. It is estimated that approximately half of the exacerbations are caused by viral or bacterial infections, such as *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* (Mc), the latter being responsible for nearly 10 % of episodes. Bacteria tend to colonize the lower airways of adults with COPD and trigger both the release of pro-inflammatory molecules and increase of the number of T cells in lung parenchyma and peripheral and central airways, thus contributing to the hallmark of COPD, i.e. the recurrent inflammation of the respiratory tract (5).

Chronic lung inflammation is followed by tissue repair and remodeling that determine the ultimate

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pathologic phenotype of COPD. The precise causes of these abnormalities in susceptible individuals, even after removal of the original noxious stimuli, remain quite mysterious (6).

Usually, when adults with COPD contract *M. catarrhalis* infection, their organism is efficiently cleared from the respiratory tract after a relatively short period (mean time, 34 days). Patients then develop strain-specific protection against re-exposure to the same bacterial strain. The majority of patients develope serum immunoglobulin G (IgG) and/or IgA responses to their isolate of *M. catarrhalis*, as demonstrated by whole-cell enzyme-linked immunosorbent assay and flow cytometry assays (7). The production of immunoglobulins specific for *M. catarrhalis* by specific plasma cells requires the activation of Mc-specific T lymphocytes. The precise role of T cells in the protective immune response and/or in the pathophysiology of COPD has not yet been elucidated. The aim of the present study is to evaluate the functional profile of Mc-specific T-cell response in the BAL of *M. catarrhalis*-infected COPD patients.

MATERIALS AND METHODS

Patients

Ten COPD patients (six males and four females; mean age, 67 years; range: 62-73 years) were recruited for the study and fully informed consent and ethics local approval were obtained. Inclusion criteria were presence of COPD (8), absence of other lung disease on the basis of the clinical assessment and absence of immunosuppressive treatments or life-threatening disorders. The diagnosis of COPD was established using the Global Initiative for Chronic Obstructive Lung Disase (GOLD) criteria (8).

Five patients had positive BAL cultures for *M. catarrhalis*, whereas the other five COPD patients had no direct evidence of *M. catarrhalis* infection (Table I).

Reagents

Recombinant human IL-2 was a kind gift from Eurocetus (Milano, Italy). Phyto-hemagglutinin (PHA) was purchased from Gibco Laboratories (Grand Island, NY), ionomycin and phorbol 12-myristate 13-acetate (PMA) from Sigma (St. Louis, MO, USA). Anti-CD3, Anti-CD4, and anti-CD8 monoclonal antibodies (mAbs) were purchased from Becton Dickinson (San Jose, CA).

Purification of outer membranes of M. catarrhalis (omMc)

The outer membranes of strains ATCC 43617 were obtained from Zwittergent extracts. Thirty chocolate agar plates were used. Bacteria were scraped from the plates into 25 ml of PBS [0.01 M sodium phosphate, 0.15 M sodium chloride (pH 7.2)] and harvested by centrifugation at 12,000 x g for 20 min at 4°C.

The bacteria were suspended in 10 ml of 1 M sodium acetate-0.001 M 3-mercaptoethanol (pH 4.0). A 90-ml volume of a solution containing 5% Zwittergent Z 3-14 (Calbiochem-Behring, La Jolla, Calif.) and 0.5 M CaCl2 was added, and the suspension was mixed for 1 h at room temperature. Nucleic acids were precipitated by the addition of 25 ml of cold ethanol and subsequent centrifugation at 17,000 x g for 10 min at 4°C. The remaining proteins were precipitated by the addition of 375 ml of cold ethanol and collected by centrifugation at 17,000 x g for 20 min at 4°C. The pellets were allowed to dry and were then suspended in 10 ml of buffer Z (0.05% Zwittergent, 0.05 M Tris, 0.01 M EDTA [pH 8.0]) and mixed for 1 h at room temperature. OMPs are present in the buffer Z soluble fraction after centrifugation of this material at 12,000 x g for 10 min at 4°C. Such an antigen prepartion is referred to as omMc. A lysate of *Helicobacter pylori* was used as control antigen. Aqueous extract of Hp was prepared by resuspending pelleted bacteria (NCTCI 1637 strain) in 2 vol and vortexing vigorously for 1 min. Bacteria and cell debris were removed by centrifugation and the protein concentration in the extract was measured using a bacterial cell lysate Ag protein assay reagent kit (Pierce, Rockford, IL).

T-cell Cloning of isolated Mononuclear Cells (MNC) from BAL

Aspirated BAL samples (2 x 50 ml) were collected and transferred to 50 ml polypropylene tubes. The first BAL specimen was processed for microbiological testing and pathogens were identified with standard techniques. The identity of an isolate as *M. catarrhalis* was confirmed by colony morphology and PCR. The second BAL specimen was centrifuged at 1,200 rpm for 15 min. The pellet was resuspended in 50 ml of PBS, layered on to Ficoll-Hypaque gradient in two 50 ml polypropylene tubes and run at 2,200 rpm for 25 minutes. At the end of centrifugation the MNC were recovered, washed, counted and resuspended in RPMI 1640 supplemented with IL-2 (20 U/ml) and 10% FCS (HyClone Laboratories, Logan, UT, USA) (complete medium).

MNC from BAL samples were cloned according to a previously described technique, which allows the clonal expansion of virtually every single T cell, regardless of its antigen specificity (9). Briefly, MNC were seeded

under limiting dilution conditions (0.3 cell/well) in round-bottomed microwells containing 10^5 irradiated autologous peripheral blood mononuclear cells (PBMC) as feeder cells and PHA (1% v/v) in a final volume of 0.2 ml complete medium. Growing microcultures were then supplemented at weekly intervals with IL-2 (30 U/ml) and 10^5 irradiated feeder cells.

Identification of omMc-specific T cells in the peripheral blood of COPD patients

To assess the presence in the peripheral blood of COPD patients of T cells specific for the outer membrane antigen(s) of *M. catarrhalis* (omMc), PBMC purified by Ficoll-Hypaque density gradient centrifugation were resuspended in medium supplemented with 3% human serum. 3 x 10^5 cells/0.2 ml were cultured in microwells for 5 days in the presence of medium, control antigen or omMc at different concentrations. Preliminary experiments showed that 0.4 μg/ml omMc was the optimal dose in all responder patients. Sixteen h before harvesting, 0.5 μCi [^{3}H]dT (Amersham Pharmacia Biotech) were added, and the radionuclide uptake was measured in a β-counter. The mitogenic index (MI) was calculated as the ratio between mean values of cpm obtained in stimulated cultures and those obtained in the presence of medium alone. MI >5 was considered as positive.

Cytofluorimetric analysis of cell surface markers and characterization of the cytokine profile of T cell clones

Cell surface marker analysis of T cells was carried out by two- or three-color flow cytometry using fluorochrome-conjugated anti-CD3, anti-CD4, anti-CD8. These cells were analyzed on a BDLSRII cytofluorimeter using the Diva software (BD Biosciences). A total of 10^4 events for each sample were acquired. To elicit cytokine production by non-specific T cell clones, T cell blasts were resuspended at 10^6/ml of complete medium and cultured for 36 h in the presence of PMA (10 ng/ml) plus anti-CD3 moAb (200 ng/ml).

To induce the cytokine production by omMc-specific T-cell clones, 10^6 T-cell blasts of each clone were co-cultured for 48 h in 1 ml medium with 5 x 10^5 irradiated autologous PBMC as antigen presentig cells (APC) with or without omMc (0.4 μg/ml). Cell-free supernatants were collected and assayed in duplicate for their IFN-γ, TNF-α, and IL-4 content (Bio-Source International, Camarillo, CA). Supernatants showing IFN-?γ, TNF-α and IL-4 levels 5 SD over the mean levels in control supernatants derived from irradiated feeder cells alone were regarded as positive. T cells clones able to produce IFN-?γ, but not IL-4 were categorized as Th1, T cell clones able to produce IL-4 but not IFN-?γ as Th2, and clones producing both IFN-γ? and IL-4 were categorized as Th0.

Preparation of B cells and immortalization of B cells with EBV

B cell-enriched suspensions were prepared by a double-step rosetting with neuraminidase-treated SRBC, as described elsewhere (9). Peripheral blood B cell-enriched suspensions usually consisted of 68 to 87% B cells, 9 to 21 % monocytes, and < 1 % T cells. They will be referred to as B cells. To obtain EBV-transformed lymphoblastoid B cell lines (EBV-B cells), B cells were incubated for 48 h with the EBV-producing marmoset cell line B95.8 supernatant and subsequently expanded in complete medium supplemented with 15% FCS.

Perforin-Mediated Cytotoxicity and Fas–Fas Ligand(L)-Mediated Apoptotic Activity

Perforin-mediated cytolytic activity of T cell clones was assessed as reported (9). Briefly, T cell blasts of Mc-specific clones were incubated at ratios of 10, 5, and 2.5 to 1 with ^{51}Cr-labeled autologous Epstein–Barr virus transformed (EBV)-B cells preincubated with omMc (1 μg/ml). After centrifugation, microplates were incubated for 8 h at 37°C, and 0.1 ml of supernatant was removed for the measurement of ^{51}Cr release (9). The ability of Mc-specific T cell clones to induce Fas–FasL-mediated apoptosis was assessed using Fas$^+$ Jurkat cells as target. T-cell blasts from each clone were cocultured with ^{51}Cr-labeled Jurkat cells at an effector/target ratio of 10, 5, and 2.5 to 1 for 18 h in the presence of PMA (10 ng/ml) and ionomycin (1 mmol/l), as reported (9). To block Fas–FasL interaction, the anti-Fas antagonistic moAb M3 (Immunex) was used at a 5 μg/ml final concentration in a 30-min pretreatment of ^{51}Cr-labeled Jurkat cells, as detailed elsewhere (9).

Assays for helper function to B cells and monocytes by T cell clones

The ability of Mc-stimulated T cell clones to induce polyclonal B cell activation was assessed by measuring [^{3}H]TdR uptake by B cells (3 X 10^4) cocultured for 4 days with irradiated (2000 rad) autologous clonal T cell blasts (3 X 10^4) in the absence or presence of omMc. The cell culture system used for the induction of Ig synthesis was performed in duplicate tubes by using complete medium supplemented with 10% FCS, as described elsewhere (9). B cells (5 x 10^4) were cultured alone or with autologous clonal T cell blasts (5 x 10^4) in the absence or presence of omMc. After 10 days, culture supernatants were collected and assayed for their Ig content. The immunoradiometric assay used for detecting IgM, IgG, and IgA has been previously described in detail (10).

To assess their ability to induce MMP-12 activity by monocytes, omMC-specific Th1 and Th0 clones were co-cultured with autologous monocytes in the presence of

medium or omMC. The MMP-12 activity was measured using the Enzolyte 490 MMP-12 Assay Kit (Anaspec, San Jose, CA).

RESULTS

Predominance of Th1 cells in the BAL of COPD patients

Among COPD patients undergoing BAL, five were BAL culture-positive for *M. catarrhalis*, and five were culture negative. Mononuclear cells were isolated from BAL and single T cells were cloned by a high efficiency cloning procedure (9). A total number of 117 $CD4^+$ and 24 $CD8^+$ T cell clones were obtained from the BAL of the Mc-positive patients, whereas 113 $CD4^+$ and 26 $CD8^+$ were the T cell clones derived from the BAL of the five Mc-negative patients (Table II). All BAL-derived T clones were assessed for their cytokine profile by measuring mitogen-induced production of IFN-γ, TNF-α and IL-4. In both Mc-positive and negative COPD patients, the majority of BAL-derived clones (mean ± SD 70 ± 12% and 72 ± 6%, respectively) were $CD4^+$ able to secrete IFN-γ and TNF-α, but not IL-4, showing thus a polarized T helper type 1 (Th1) profile (Fig. 1). $CD4^+$ Th0 clones able to secrete both IFN-γ and IL-4 accounted for only 18 ± 5% and 15 ± 3% of clones from Mc-positive and Mc-negative patients, respectively. Likewise, in both series of patients, the proportions of cytotoxic $CD8^+$ clones producing type 1 cytokines (Tc1 clones) (10 ± 5% vs. 6 ± 3% and 12 ± 4% *vs* 5 ± 2%, respectively) were relatively higher ($p = 0.0401$ and $p = 0.0109$) than those of $CD8^+$ clones producing both type 1 and 2 cytokines (Tc0 clones) (Fig. 1).

M. catarrhalis-specific T cells in the BAL of Mc-positive COPD patients

BAL-derived T cell clones from COPD patients were assayed for proliferation in response to omMc. None of the 50 $CD8^+$ clones derived from the BAL of Mc-positive or Mc-negative patients showed proliferation to that antigen preparation. In contrast, 30 (26%) of the 117 $CD4^+$ T cell clones generated from BAL T cells of Mc-positive patients proliferated to omMc, but not to control antigen (*H. pylori* lysate). Under the same experimental conditions, none of the 113 $CD4^+$ T clones obtained from the BAL of Mc-negative patients showed significant proliferation to the same antigen preparation (Table II).

Th1 effector functions of BAL-derived M. catarrhalis-specific T cells

We then assessed the cytokine profile induced in Mc-specific BAL-derived T cell clones by stimulation with omMc in the presence of irradiated autologous APCs. The majority of omMc-specific Th clones produced Th1 cytokines upon stimulation with the specific antigen (Fig. 2). Since most antigen-activated Th1 and Th0 clones express perforin-mediated cytotoxicity against autologous APC (e.g., antigen-pulsed B cells) (9), we assessed the cytolytic potential of omMc-specific T cell clones by using antigen-pulsed ^{51}Cr-labeled autologous EBV-B cells as targets. At an effector to target ratio of 10:1, all 23 Th1 and 5 out of 7 Th0 clones lysed omMc-presenting autologous EBV-B cells (Fig. 3A). Because activated effector Th cells can also kill their targets by inducing apoptosis through Fas–FasL interaction, we evaluated the ability of activated omMc-specific T-cell clones to induce ^{51}Cr release by Fas^+ Jurkat cells undergoing apoptosis. Upon mitogen activation, 21 of 23 Th1 (91%) and 4 out of 7 Th0 clones were able to induce apoptosis in target cells (Fig. 3B). The role of Fas–FasL interaction in the ^{51}Cr release was confirmed by its inhibition (range 39.6–59.8%) using a blocking anti-Fas antibody (data not shown).

omMc-specific Th clones express Ag-dependent help for Ig production

The ability of all omMc-specific T cell clones to provide B-cell help was then investigated. To this end, irradiated T cell blasts were cocultured at 1:1 ratio with autologous B cells in the absence or presence of omMc or control antigen. B cell proliferation was measured on day 4. In parallel experiments, T cell blasts were cocultured at 1:1 ratio with autologous B cells in the absence or presence of omMc and IgM, IgG, and IgA levels were measured in cell-free culture supernatants on day 10. In the presence of medium alone or control antigen, neither B cell proliferation nor increase in IgM, IgG, and IgA production above the spontaneous levels measured in cultures containing B cells alone were observed. In the presence of omMc, all 30

Table I. *Clinical presentation of COPD patients.*

Patients	Age/ sex	GOLD Diagnosis	Years since Diagnosis	Smoker	BAL Cultures
L. M.	62/F	Moderate COPD	*8*	*yes*	*M. catarrhalis*
A. E.	73/M	Severe COPD	*15*	*yes*	*M. catarrhalis*
M.D.	65/F	Severe COPD	9	*no*	*M. catarrhalis*
S. G.	70/M	Moderate COPD	12	*yes*	*M. catarrhalis*
L. D.	62/M	Severe COPD	10	*yes*	*M. catarrhalis*
M. L.	63/M	Severe COPD	*8*	*no*	*H. influenzae*
B. I.	70/F	Moderate COPD	*13*	*yes*	*S. pneumoniae*
L. S.	64/M	Severe COPD	*9*	*yes*	*H. influenzae*
M. F.	66/M	Severe COPD	*10*	*yes*	*H. influenzae*
B. A.	73/F	Moderate COPD	*16*	*yes*	*S. pneumoniae*

specific clones provided substantial help for B cell proliferation, the mean mitogenic index being 21 (range 8-43). The omMc-specific Th clones from BAL were also efficient in providing help for IgM, IgG, and IgA antibody production by autologous B cells (Fig. 4).

BAL-isolated T cells help monocyte MMP-12 production

Antigen-stimulated omMc-specific T cell clones from the BAL of Mc-pos patients were co-cultured with autologous monocytes. Apart from six Th0 and three Th1 clones that provided poor or no helper function, in 43 omMC-specific clones, antigen stimulation resulted in the expression of substantial help for MMP-12 activity by monocytes (Fig. 5).

Presence of omMc-specific T lymphocytes in the peripheral blood of M. catarrhalis-positive COPD patients

In order to assess the presence of T cells specific for omMc in the peripheral blood of COPD patients, PBMC isolated from all ten patients and five healthy subjects were re-suspended in medium-3% human

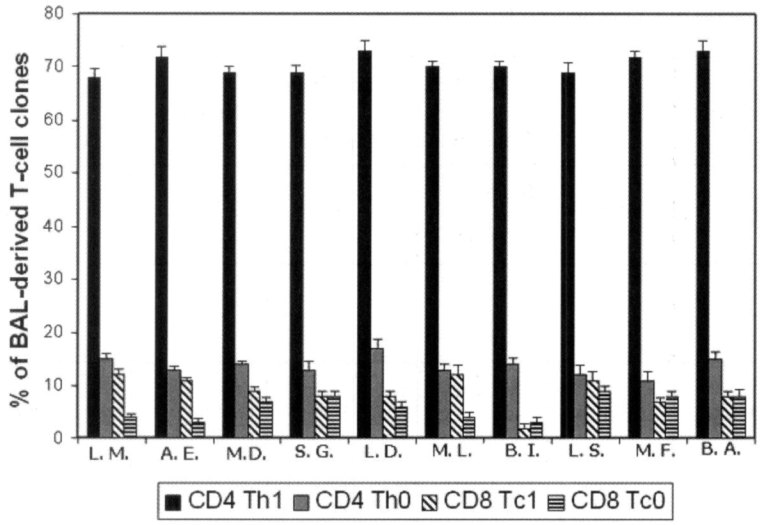

Fig. 1. *Cytokine profile of the T cell clones derived from BAL. BAL-derived T cell clones were obtained from 10 COPD patients, the first five (L.M., A.E., M.L., S.G., L.D.) were BAL positive for Moraxella catarrhalis, whereas the other five (M.D., B.I., L.S., M.F., B.A.) negative for Moraxella catarrhalis. Duplicate samples of supernatants of mitogen-stimulated T cell clones were assayed for cytokine production. $CD4^+$ and $CD8^+$ clones able to produce IFN-γ, but not IL-4, were categorized as Th1 and Tc1, whereas $CD4^+$ and $CD8^+$ clones producing both IFN-γ and IL-4 were coded as Th0 and Tc0, respectively. Results represent mean percentage proportions (±SD) of clones with the indicated cytokine profile.*

serum. Cells (3 x 10^5) were cultured for 5 days in the presence of medium, control antigen or omMc (0.4 μg/ml). As shown in Fig. 6, only PBMC of patients with COPD and positive for *M. catarrhalis* proliferated in the presence of omMc. These data demonstrate that omMc-specific T lymphocytes do recirculate from the site of infection to the periphery and can be detectable in the peripheral blood of *M. catarrhalis*-positive patients, but not in the blood of *M. catarrhalis*-negative patients. This simple assay able to assess the T cell-mediated immunity to omMc may represent an useful diagnostic tool in COPD patients.

DISCUSSION

In this study we demonstrate the presence of T cells specific for *M. catarrhalis* antigens in the BAL and peripheral blood of COPD patients infected with *M. catarrhalis*. The clonal progeny of T cells present in BAL samples had the ability to secrete IFN-γ and TNF-α, whereas the concomitant production of Th2 cytokines was limited to a few clones. Such a cytokine pattern was present in the BAL of both *M. catarrhalis*-positive and *M. catarrhalis*-negative COPD patients.

The possibility that this pattern does not reflect the real functional behaviour of airway-infiltrating T cells *in vivo*, but is the result of *in vitro* artifacts due to culture in IL-2 and cloning procedures, was investigated. However, using the same culture and cloning protocol, T cell clones with predominant Th2 profile were obtained from bronchial mucosa of atopic patients (11).

Our *in vitro* findings are in agreement with the *ex vivo* data indicating a preferential expression of IFN-γ and IFN-γ inducible CXC chemokines in the bronchial mucosa (12-13). Since CXC chemokines attract cells equipped with CXCR3 receptor, a membrane molecule preferentially expressed by type 1 T cells (14), the *in situ* release of those chemokines may explain why the Th1 is the predominant functional profile of bronchial mucosa-infiltrating T cells.

Several data confirmed a prevalent Th1/Tc1 immunosurveillance in COPD: the chemokine receptor CCR5, preferentially expressed by T cells producing IFN-γ, is increased in mild/moderate

Table II. *Antigen specificity of T cell clones isolated from BAL fluids. $CD4^+$ and $CD8^+$ clones were tested for proliferation to outer membranes of M. catarrhalis (omMc) in the presence of irradiated autologous APC. No $CD8^+$ clones significantly proliferated to omMC.*

Patients and source of T cells	Total number of $CD4^+$ T cell clones obtained	Number (%) of $CD4^+$ clones reactive to omMC
BAL from Mc-positive patients		
L.M.	38	9 (24)
A.E.	20	5 (25)
M.D.	18	5 (28)
S.G.	22	6 (22)
L.D	19	5 (26)
All cases	*117*	*30 (26)*
BAL from Mc-negative patients		
M.L.	17	0
B.I.	14	0
L.S.	36	0
M.F.	20	0
B.A.	26	0
All cases	*113*	*0*

disease (15). The expression of the transcription factor signal transducer and activator of transcription (STAT)-4 is also critical for the differentiation of Th1/Tc1 cells (16). Increased STAT-4 nuclear expression in bronchial epithelium and submucosa of mild/moderate COPD patients in comparison with controls has been reported (17).

M. catarrhalis infection has been implicated in the COPD and in particular in the exacerbations of the disease (18), although its specific contribution to the pathogenesis of COPD has recently been questioned (19-20). We have observed that subjects who contracted infection with *M. catarrhalis* display not only humoral (7) but also T cell-mediated responses to the pathogen at the site of the infection (BAL) and in the periphery. It is of note that *M. catarrhalis*-specific T cells are recruited in the bronchial mucosa, where they reside in an activation state near the source of their specific antigen(s). This situation offers the opportunity for both proliferation and expression of their Th1 effector functions.

One can speculate that the cytokines produced *in situ* by specific T lymphocytes stimulate macrophages and bronchial cells to produce proteins and cytokines able to change the bronchial architecture and functionality (21). There are a many inflammatory factors that contribute to the various mechanisms of airways remodeling and, in particular, IFN-γ accelerates airway hyperresponsiveness (22) and the Th1 cell-induced mucus hyperproduction (23). TNF-α stimulates airways smooth muscle proliferation and production of GM-CSF that plays

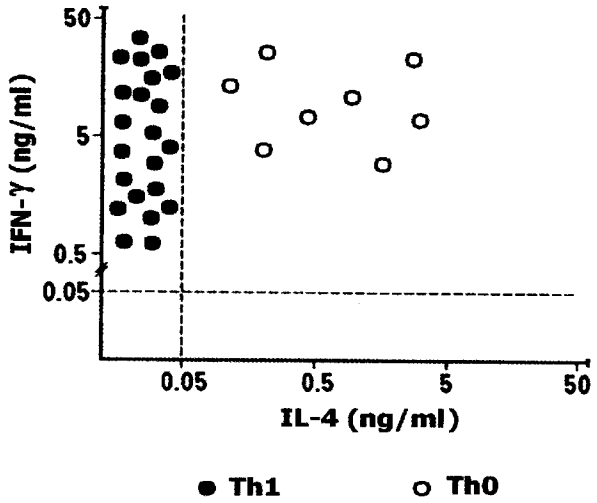

Fig. 2. *Moraxella catarrhalis antigen-induced cytokine production by T cell clones. MC-specific Th1, Th0 clones were stimulated with the appropriate antigen, and IFN-γ, and IL-4 production was measured in culture supernatants. In unstimulated control cultures, levels of IFN-γ and IL-4 were consistently <0.05 ng/ml.*

a role in promoting both recruitment and survival of eosinophils (24).

Hogg et al. (25) provided a quantitative cellular analysis of lung tissue samples from patients with COPD of all degrees of severity and lung function impairment. The presence of lymph follicles in the parenchyma or of dendritic and Th1 cell infiltration was noted, raising questions about the nature of the immune response and its potential role in emphysematous destruction. A pathogenetic model of emphysema suggests that neutrophil elastase and matrix metalloproteinase MMP-12 released by activated macrophages enzymatically destroy the elastin scaffold of the alveolar spaces. However, the production of proteases is not restricted to inflammatory cells; also structural cells, such as epithelial and endothelial cells, can produce proteases (26). Strong support to this concept is provided by data showing that elimination of alveolar macrophages in rats (27) or deletion of MMP-12 gene in mice (28) protected against induced emphysema.

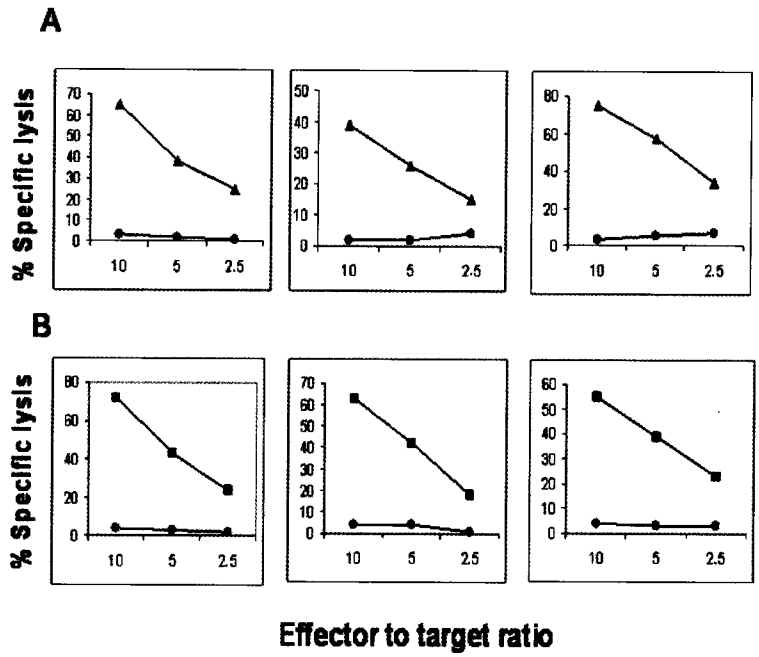

Fig. 3. *Cytotoxic and proapoptotic activity of Moraxella catarrhalis-specific T cells isolated from the BAL. (A) To assess their perforin-mediated cytotoxicity, MC-specific T cell clones were cocultured at different effector-to-target ratios with ^{51}Cr-labeled autologous EBV-B cells pulsed with outer membranes of M. catarrhalis (▲) or lysate of Helicobacter pylori (●), and ^{51}Cr release was measured as index of specific target cell lysis. (B) To assess their ability to induce apoptosis in target cells, MC-specific T cell clones stimulated with mitogen (■) or medium alone (●) were cocultured with ^{51}Cr-labeled Fas^{+} Jurkat cells, and ^{51}Cr release was measured as index of apoptotic target cell death. Data of three representative clones are reported.*

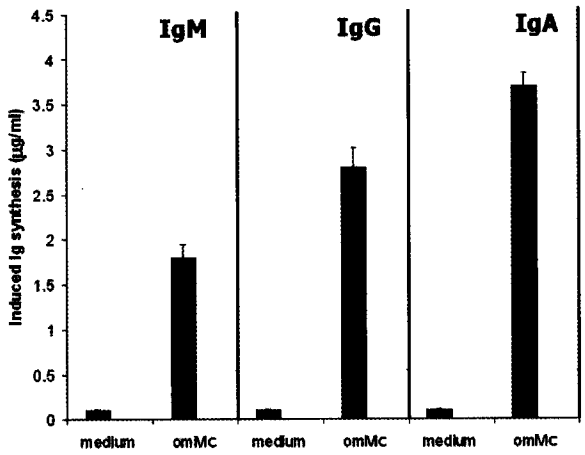

Fig. 4. *In vitro synthesis of IgM, IgG, and IgA induced in autologous B cells by Mc-specific CD4⁺ T cell clones stimulated with the specific Ag. T cell blasts from each clone (5 x 10⁴) were cultured with autologous B cells (5 x 10⁴) in the presence of the outer membranes of Moraxella catarrhalis. After 10 days, cell-free culture supernatants were assayed for their Ig content by appropriate irnmunoradiometric assays, as previously described (9). The results represent the mean (± SE) Ig levels induced by T cell clones over the spontaneous Ig production in cultures of B cells alone.*

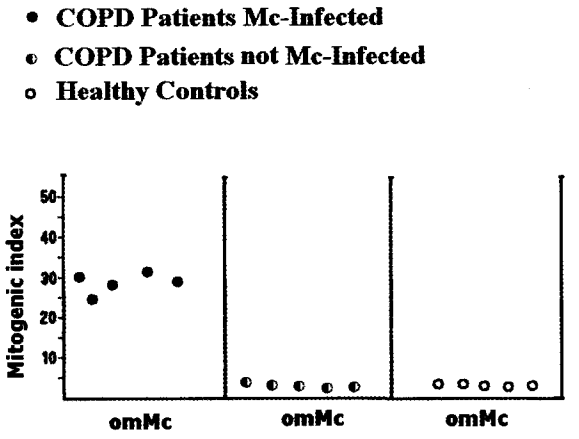

Fig. 6. *Proliferation of the PBMCs of COPD patients and healthy controls in presence of outer membranes of M. catarrhalis (0.4 µg/ml). The mitogenic index (MI) was calculated as the ratio between mean values of cpm obtained in stimulated cultures and those obtained in the presence of medium alone.*

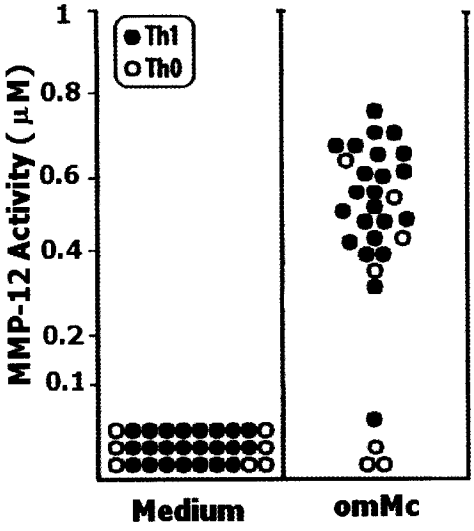

Fig. 5. *omMc-specific T cells induce MMP-12 activity by monocytes. To assess their ability to induce MMP-12 activity by monocytes, omMC-specific Th1 and Th0 clones were co-cultured with autologous monocytes in the presence of medium or omMC, and MMP-12 activity was measured using the Enzolyte 490 MMP-12 Assay Kit (Anaspec, San Jose, CA).*

Further studies are required to investigate whether the cytokine milieu generated by activated T cells may enable "non-professional" APCs, such as smooth muscle or epithelial cells, to present antigens available in the airways tissue to infiltrating T cells and becoming target of their cytolytic and proapoptotic activity, a functional property found in more 90% of the omMc-specific T clones isolated from BAL of *M. catarrhalis*-positive COPD patients.

Recent data from both human and animal studies of COPD (29.30) suggest that a further mechanism might be involved in the pathogenesis of COPD: disruption of the balance between apoptosis and replenishment of structural cells in the lung might contribute to the destruction of lung tissue in response to inflammation stimuli, leading to emphysema. It has been suggested that T-cell inflammation would be a response to the disturbance of the balance between apoptosis and regeneration

of structural lung cells (31). An important result of this study is that the *M. catarrhalis*-specific T cells provided helper activity for the production of immunoglobulins by autologous B cells, and these data are in agreement with previous data showing the presence of Mc-specific antibodies in Mc-infected patients (7).

It is currently believed that excessive inflammatory response to inhaled irritants, e.g. cigarette smoke, causes progressive airflow limitation, attraction of macrophages and neutrophils by chemokines, leading to oxidative stress, emphysema, small airways fibrosis, and mucus hypersecretion. Our findings support the hypothesis that a component of COPD is represented by T cell-mediated immune responses to *M. catarrhalis* or other pathogens (e.g *Streptococcus pneumoniae* or *Haemophilus influenzae*), that may be inappropriate or even pathogenic in terms of time of onset, intensity, and target. In other words, COPD would be the outcome of a Th1-driven immunopathological process that, in combination with other mechanisms, would result in lung damage and remodelling. Support to this hypothesis about the critical role of Th1 cells and IFN-γ in the pathogenesis of COPD is provided by the model of Th1-prone C57BL/6 mice, that develops an inflammatory emphysematous and fibrotic phenotype (32) and by the model of mice immunised with xenogeneic human umbelical vein endothelial cells (HUVEC) (33), in which intraperitoneal injection of endothelial cells causes emphysema, activation of MMP-2 and MMP-9, and generation of anti-endothelial cell antibodies. This model provides evidence for a T cell-mediated mechanism of endothelial cell apoptosis in the development of emphysema, because emphysema can be induced by passive transfer of $CD4^+$ T cells to naive recipient animals (34).

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