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Integrin αEβ7 expression on BAL CD4+, CD8+, and γδ T-cells in bleomycin-induced lung fibrosis in mouse

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ABSTRACT: CD4, CD8, and γδ T-cells located in the epithelium express the integrin αEβ7 that binds to E-cadherin on the epithelium. γδ T-cells mediate specific cellular immune functions and can recognize damaged cells directly. It was, therefore, of interest to analyse the presence of γδ T-cells and the expression of αEβ7 on γδ T-cells in the bleomycin (BLM) model of pulmonary fibrosis.

Lung fibrosis was induced by a single intratracheal instillation of BLM (0.125 U·mouse^{-1}), and bronchoalveolar lavage (BAL) T-cell subpopulations were examined at various time-points for the expression of the integrin αEβ7 by flow cytometry.

CD4+ T-cells accounted for about 40% of the lymphocytes, compared to about 10% of CD8+ T-cells and 10–14% γδ T-cells. Within the CD4+ T-cell population the proportion of αEβ7+ cells decreased between Days 2 and 22 from 36 to 11%. The percentage of αEβ7+ CD8+ T-cells increased at the same time from 4 to 68%. However, more than 80% of the γδ T-cells in BAL fluid expressed αEβ7 at all time-points. The surface-expression of this integrin on γδ T-cells was 2–3 times higher than on CD4+ or CD8+ T-cells.

This predominant expression of αEβ7 on γδ T-cells suggests a role for these cells in the pathogenesis of bleomycin-induced lung fibrosis.

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Bleomycin (BLM) represents a group of glycopeptides that are used as chemotherapy in the treatment of cancer. The use of BLM as an antineoplastic drug is, however, limited because it produces a dose-dependent pneumonitis, which often progresses to interstitial pulmonary fibrosis in humans [1]. Interstitial pulmonary fibrosis is accompanied by an overexuberant repair process, which is characterized by an excessive number of fibroblasts, an absolute increase in lung collagen content, and abnormality in the ultrastructural appearance and spatial distribution of collagen types [2]. Intratracheal instillation of BLM in rodents is often used to model interstitial pulmonary fibrosis, as it occurs in humans [3], and is similar to other models of fibrosis. It is thought that the general events that lead to fibrosis include the initial damage, development of alveolitis and inflammation, and, subsequently, excessive accumulation of collagen in the lung.

Integrins promote highly stabilized adhesion to extracellular matrix (ECM) proteins and counter-receptors on other cells [4]. They are composed of noncovalently associated heterodimeric glycoprotein subunits α and β. At present, 17 α and 8 β subunits have been identified in vertebrates. Some integrins, such as the β2 integrins lymphocyte function associated antigen-1 (LFA-1) (αLβ2), Mac-1 (αMβ2), and p150,95 (αXβ2), are expressed exclusively on leucocytes, whereas other integrins, such as members of the β1 subfamily, are expressed both on leucocytes and on other cells. Several members of the β1 subfamily of integrins are expressed on T-lymphocytes either constitutively or after activation.

Another subfamily of integrins, the β7 subfamily, is expressed on the surface of lymphocytes, particularly in the mucosal immune system [5, 6]. The mucosal immune system includes lymphocytes located in the gut, oral, nasal, pulmonary and genitourinary tracts, as well as the mammary gland. One member of the β7 subfamily is composed of β7 in association with α4, an α-subunit that also pairs with β1. The other known β7 integrin, composed of the β7 chain in association with an α-subunit designated αE or αHML-1, mediates binding to the basolateral side of the epithelium to E-cadherin [7, 8].

Most T-cells recognize antigen *via* a clonally distributed CD3-associated T-cell receptor (TCR) αβ heterodimer. In recent years, a CD3+ subset of lymphocytes bearing an alternative TCR, called γδ was identified [9]. In contrast to mature αβ cells, the majority of γδ T-cells do not express either CD4+ or CD8+, although a minor subset of CD8+ γδ T-lymphocytes can be identified [10, 11].

We have analysed cellular changes in mice during the first 30 days after intratracheal BLM instillation. The monoclonal antibody M290 [7, 12] to the mouse αEβ7 integrin was used to analyse the expression of this molecule on T-lymphocytes infiltrating the lung. We found that CD4+, CD8+, natural killer (NK), and γδ T-cells infiltrate the lung in response to BLM instillation into the lung. In addition, we observed that the integrin αEβ7

is expressed on some bronchoalveolar lavage (BAL) fluid CD4+ and CD8+cells but is expressed by the majority of γδ T-lymphocytes infiltrating the lung airways.

Methods

Animals

All experiments were carried out in male albino Swiss-Webster mice weighing 28–30 g (Simonsen, Gilroy, CA, USA). Animals were caged in groups of four in Animal Resource Services facilities approved by the American Association for the Accreditation of Laboratory Animal Care, and were allowed to acclimatize for 1 week before the start of each experiment. Cages were maintained in laminar flow hoods to minimize pulmonary infections. To induce pulmonary fibrosis, mice were anaesthetized with Vetalar (ketamine HCl) / xylazine (43.3 and 14.7 $mg \cdot kg^{-1}$, respectively) and treated on Day 0 with 0.125 units bleomycin sulphate (generously donated by the Bristol-Myers Company, Syracuse, NY, USA) in 50 µL sterile isotonic saline by intratracheal instillation, as described previously [13]. At the days indicated, animals were anaesthetized with pentobarbital (120 $mg \cdot kg^{-1}$ *i.p.*) and killed by exsanguination.

Bronchoalveolar lavage

Lung lavage was performed as described previously [14]. Briefly, after cannulation of the trachea, the lungs were lavaged with 4 × 1 mL phosphate-buffered saline (PBS) without Ca^{2+}/Mg^{2+}. The volume of the recovered fluid was determined and ranged 3.2–3.6 mL. The fluid was centrifuged at 280×g for 7 min at 4°C. An aliquot of the supernatant was frozen for protein determination. The remaining fluid was pooled at each time-point for cytokine determination. The cell pellet was washed in PBS and resuspended in PBS with 1% bovine serum albumin (BSA). The cells were counted with a Coulter counter (Coulter Electronics Inc., Hialeah, FL, USA) and 10^5 cells were dispersed on slides using a Cytospin (Shandon Inc., Pittsburgh, PA, USA).

Hydroxyproline assay

The lungs of all animals were used to assay for hydroxyproline content after BAL. Lungs were perfused *in situ* through the right ventricle with ice cold isotonic saline (6 mL). The lobes were quickly dissected free of non-parenchymal tissue and frozen at -80°C. Later, the frozen lungs were thawed and homogenized in PBS without Ca^{2+}/Mg^{2+}. The final volume of the homogenate was adjusted to 5 mL. Two hundred and fifty microlitres of 50% trichloracetic acid (TCA) was added to 1 mL of the homogenate. After 10 min on ice, samples were centrifuged, the supernatant discarded, and the precipitate hydrolysed in 2 mL of hydrochloric acid (6 N) overnight (16 h) at 110°C. To monitor recovery, tritiated hydroxyproline (1.0×10^5 dpm) was added to each hydrolysed sample. The hydroxyproline content of the sample was measured by the technique of WOESSNER [15]. The recovery of tritiated hydroxyproline ranged 85–98%, and it was used to correct the amount of hydroxyproline for each sample.

Antibodies

Monoclonal antibodies used for fluorescence-activated cell sorter (FACS) staining against CD4 (clone H129.19), and CD8 (clone 53-6.7) were purchased from Gibco BRL Life Technologies Inc. (Gaithersburg, MD, USA); anti-CD3 (clone 145-2C11), anti-γδ T-cell receptor (clone GL3), and anti-NK cell antibodies (clone PK1 36) were purchased from Pharmingen (San Diego, CA, USA). Control antibodies (rat immunoglobulin G (IgG)) and goat-anti-rat antibodies was purchased from Cappel/Organon Teknika Corp. (Durham, NC, USA). Cell supernatant containing the antibody M290 was prepared as described previously [12].

Analysis of leucocytes and lymphocyte subsets

BAL leucocyte count was determined in a Coulter counter and results were expressed as total number of cells per millilitre of recovered fluid. Cytological examination of cells in BAL fluid was performed after cytocentrifugation and staining with LeukoStat (Fisher Diagnostics, Orangeburg, NY, USA), a modification of the Wright's Stain technique. The relative proportions of the various leucocyte subpopulations were determined by a cell differential count of 200 cells. Specific binding of monoclonal antibody (MoAb) was analysed by direct immunofluorescence according to standard methods recommended by Becton-Dickinson Monoclonal Center (Mountain View, CA) using a cytofluorograph (FACScan, Becton-Dickinson,). Briefly, 50 µL of cell suspension (1×10^5 cells) was incubated in the presence of saturating concentrations of fluorescein- or phycoerythrin-conjugated MoAb in the dark on ice for 30 min. Erythrocytes were lysed by adding 3 mL lysing solution (0.155 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM ethylenediamine tetra-acetic acid (EDTA), pH 7.3) for 3–5 min. Leucocytes were washed twice with PBS containing 1% BSA and 0.1% sodium azide. Cytofluorimetric analysis was performed with scatter gates set on the lymphocyte fraction by forward and side scatter or side scatter (SSC) and PE fluorescence FL2 using laser excitation at 488 nm. The number of immunofluorescence-positive cells was determined out of 10,000 cells analysed. Specific binding of MoAbs was controlled by subtraction of isotype-matched rat immunoglobulins.

Statistical analysis

Quantitative data were expressed as the mean±SEM or as mean±SD. To compare the data between different time-points the Wilcoxon signed ranks test was used. Two-sided t-test p-values were calculated, and p-values of less than 0.05 were considered significant. Calculations were performed on a Macintosh computer using Systat for Macintosh, Version 5.2 (Systat Inc., Evanston, IL, USA).

Results

Lung hydroxyproline content after BLM instillation

The hydroxyproline content of the lung serves as an index of collagen accumulation. Significant accumulation of collagen in the lungs of treated mice started at Day 7 and peaked at Day 22 after BLM instillation (table 1). The hydroxyproline content was significantly higher ($p<0.05$) at Days 10, 15 and 22 after BLM instillation compared to controls at Day 0. Significantly lower values were seen at Days 2 and 4.

Changes in the leucocyte subpopulations after BLM instillation

In normal lungs (Day 0), 99% of the cells recovered by BAL were macrophages (table 2). BLM induced a massive influx of neutrophils starting at Day 2 and peaking between Days 7 and 15 after BLM instillation. Following BLM instillation, the number of macrophages did not change substantially until Day 10 but was subsequently followed by a significant increase. Lymphocytes were barely detected in the BAL fluid of control lungs but were already found in BAL fluid at Day 2 after BLM instillation. A significant increase in lymphocyte number was noted at Day 10, with highest numbers at Day 15.

Table 1. – Effects of a single intratracheal instillation of bleomycin (0.125 U·mouse^{-1}) on lung hydroxyproline at different times

Time after BLM instillation days	Hydroxyproline µg·lung^{-1}
0	310±10
2	285±7
4	274±12
7	370±30
10	506±43*
15	531±41*
22	711±75*
30	560±93

Each value represents the mean±SEM of four mice at each time-point. BLM: bleomycin. *: significantly higher ($p<0.05$) than at Day 0.

T-lymphocyte subpopulations after BLM instillation

The total number of T-cells in the BAL fluid increased between Days 2 and 4 (fig. 1a and b) and increased dramatically after Day 10. CD4+ cells represented the largest T-cell population in BAL fluid (fig. 1a and b). The evaluation of lymphocyte subsets in BAL fluid showed that the relative number of CD8+ T-cells, NK cells and γδ T-cells did not show big variations during the study period, whereas the relative number of CD4+ T-cells was enhanced at Days 15 and 22 (fig. 1b). γδ T-cells were found in the BAL fluid throughout the whole experiment, comprising 6–15% of the lymphocytes.

Analysis of integrin αEβ7 expression

Cytofluorimetric analysis of the expression of αEβ7 on CD4+, CD8+ and γδ T-cells was performed with scatter gates set on the lymphocyte fraction by side scatter and FL2. The histograms in figure 2 show the expression of the integrin αEβ7 on CD4+, CD8+ and γδ T-cells at Day 10. Similar analysis was performed for all the other time-points of the experiment (histograms not shown, data are shown in figures 3 and 4).

Expression of integrin αEβ7 on BAL T-lymphocytes

After BLM instillation, the relative number of CD8+ T-cells in BAL fluid staining for M290 increased between Days 2 and 22 after *i.t.* instillation of BLM (fig. 3b). More than 80% of the γδ T-cells in BAL fluid expressed the integrin αEβ7. The absolute number of T-cells expressing αEβ7 was found to be unchanged between Days 4 and 7 (fig. 3a), but it increased substantially after Day 10 and peaked at Day 15, without any significant difference between CD4+, CD8+ and γδ T-cells. The γδ T-cells in BAL fluid show very high expression of αEβ7 compared with the CD4+ and CD8+ T-cells (figs. 2 and 4) as determined by FACS-mode

Table 2. – Effect of a single intratracheal instillation of BLM (0.125 U·mouse^{-1}) on differential cell counts of BAL fluid at varying times

Time after BLM instillation days	Leucocytes in BAL cells ×10^3·mL^{-1}			
	Macrophages	Neutrophils	Lymphocytes	Eosinophilic neutrophils
0	16.2±2.7	0.08±0.01	0.16±0.03	0
2	17.5±3.0	5.5±0.9	0.24±0.04	0.24±0.04
4	21.2±3.7	15.2±2.6	2.3±0.4	0.26±0.05
7	11.9±1.0	29.6±2.5*	1.9±0.2	0.8±0.1
10	19.6±1.5	30.9±2.4*	7.5±0.6*	0.8±0.1
15	50.2±8.2*	29.8±4.9*	32.1±5.2*	3.9±0.6
22	128.2±33.2*	19.1±4.9	19.3±5.0*	8.0±2.1
30	79.0±14.4*	1.14±0.21*	5.0±0.9*	0.6±0.1

Counts are presented as mean±SEM of four animals at each time-point. BLM: bleomycin; BAL: bronchoalveolar lavage. *: significantly different from Day 0 values ($p<0.05$).

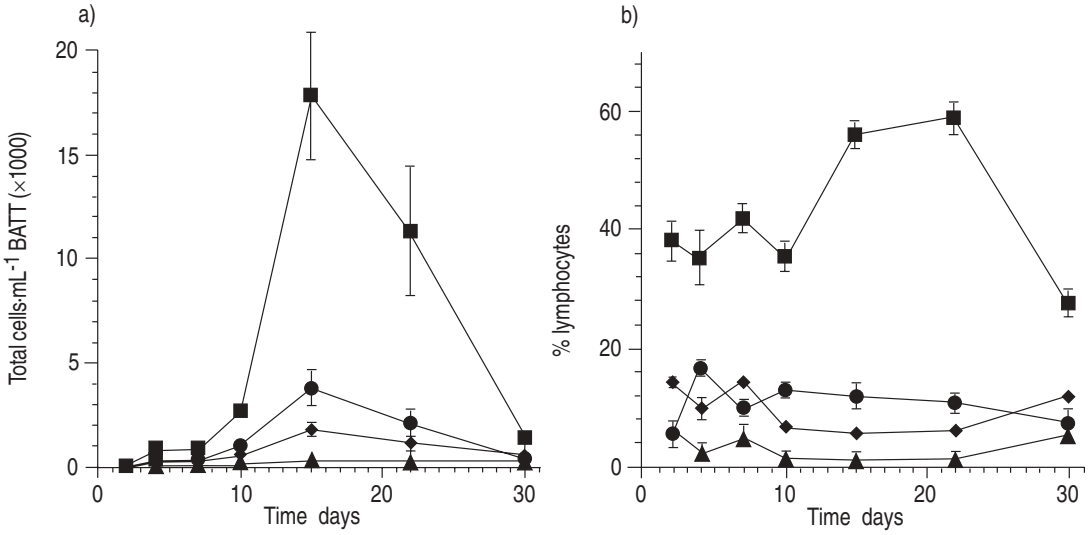

Fig. 1. – Effect of intratracheal instillation of a single dose of BLM (0.125 U·mouse^{-1}) on: a) the total number; and b) the proportion of CD4+, CD8+, NK+ or γδ T-cells in BALF. a) the values indicate the cell number·mL^{-1} BALF ±SEM; and b) the values indicate the relative number±SD within the gated lymphocyte population. —■— : CD4+; —▲— : NK; —●— : CD8+; —◆— : γδ T-cells. BLM: bleomycin; BALF: bronchoalveolar lavage fluid; NK: natural killer.

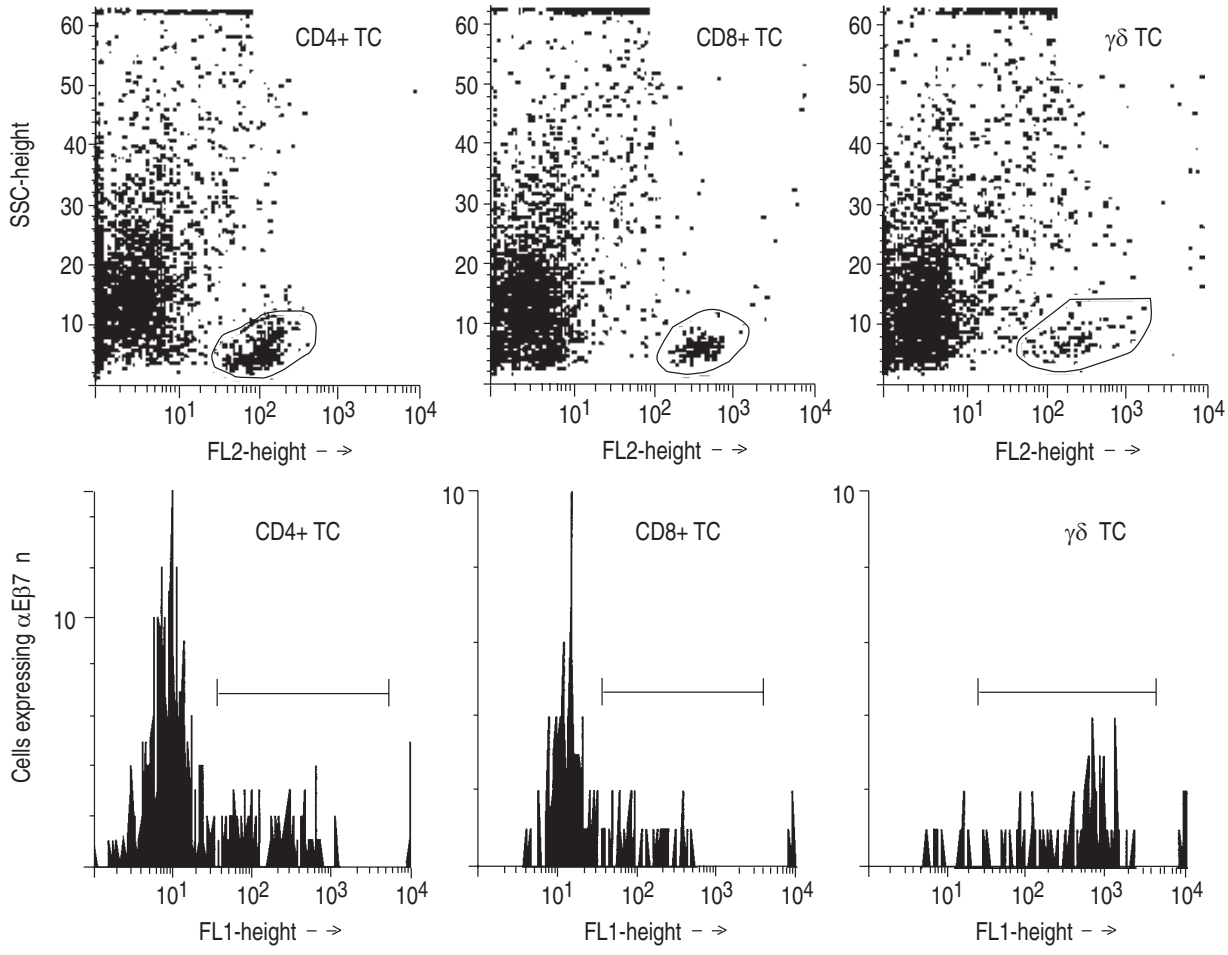

Fig. 2. – Cytofluorimetric analysis of the expression of αEβ7 on CD4+, CD8+, and γδ T-cells. Pooled BAL fluid cells of four animals at each time-point were labelled with the antibody M290 to αEβ7, then stained with FITC-conjugated goat-anti-rat IgG and double-stained with PE labelled antibodies to CD4+, CD8+, or γδ T-cells, respectively. Cytofluorimetric analysis was performed with scatter gates set on the lymphocyte fraction by side scatter (SSC) and PE fluorescence (FL2) shown in the upper panels. The histograms in the lower panels show the expression of the integrin αEβ7 on CD4+, CD8+, and γδ T-cells at day 10 with the gate (horizontal bar) shown for positive stained cells. Similar analysis was performed for all the other time-points of the experiment. TC: T-cells; BAL: bronchoalveolar lavage; FL1: fluorescin isothiocyanate fluorescence; PE: phycoerythrin; Ig: immunoglobulin G; FL: fluorescence.

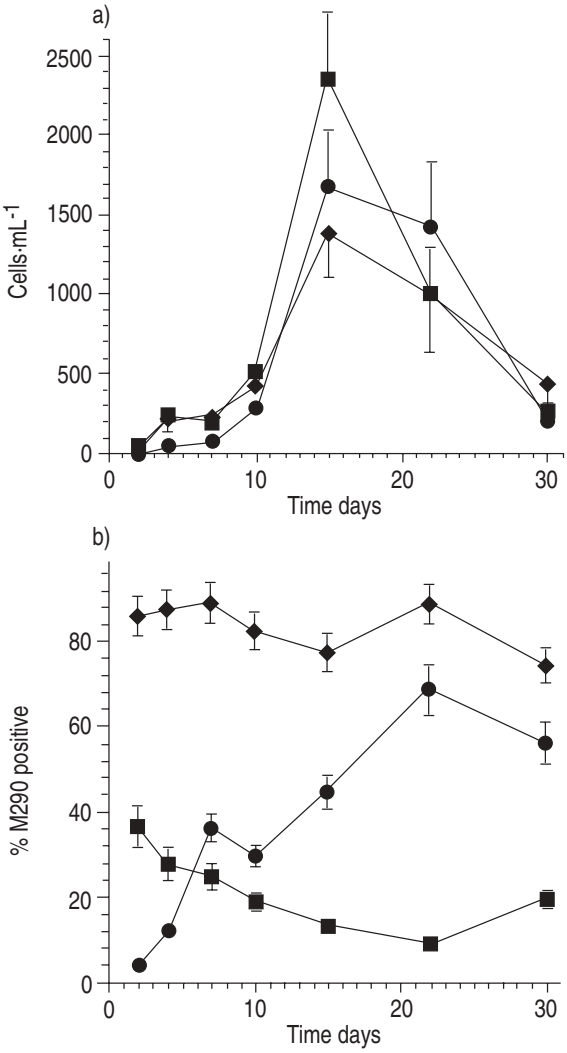

Fig. 3. – Expression of the integrin αEβ7 on CD4+, CD8+ and γδ T-cells in BALF after intratracheal instillation of a single dose of BLM (0.125 U·mouse^{-1}). a) The number ±SD of M290+ and CD4+, CD8+, or γδ T-cells in BALF in cells·mL^{-1}. b) The proportion ±SD of M290+ lymphocytes within the different T-cell subpopulations. —■—: CD4+; —●—: CD8+; —◆—: γδ T-cells. For further experimental details and abbreviations see legend to figures 1 and 2.

values. The relative logarithmic mean fluorescence (LMF) of γδ T-cells stained for αEβ7 was found to exceed the values seen on CD4+ and CD8+ T-cells by three- to four-fold. Increasing values were detected for CD4+ T-cells after Day 15, whereas CD8+ T-cells showed low values at Days 10 and 15, with similar values at all other time-points.

Discussion

Intratracheal instillation of BLM in mice induces pulmonary fibrosis characterized by collagen deposition and inflammatory cell infiltration. Increased numbers of lymphocytes in the BAL fluid were found shortly after the instillation, where about 40% of the lymphocytes accounted for CD4+ T-cells, about 10% for CD8+ T-cells and 10–14% for γδ T-cells. Within these T-cell populations, the proportion of αEβ7+ cells was over 80% in the γδ T-cell population at all time-points and

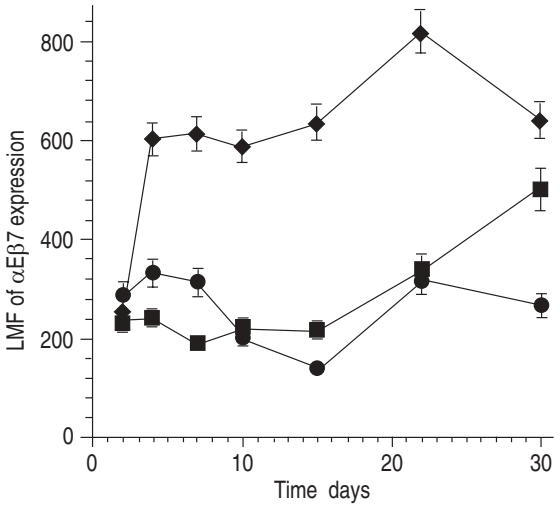

Fig. 4. – Intensity of αEβ7 expression on BALF CD4+, CD8+, and γδ T-cells at different time-points after intratracheal instillation of a single dose of BLM (0.125 U·mouse^{-1}). The values are given as the logarithmic mean fluorescence (LMF) ±SD of the αEβ7 expression on CD+, CD8+ and γδ T-cells in BALF expressing αEβ7. —■—: CD4+; —●—: CD8+; —◆—: γδ T-cells. BLM: bleomycin; BALF: bronchoalveolar lavage fluid.

the surface-expression of this integrin on γδ T-cells was 2–3 times higher than on CD4+ or CD8+ T-cells.

The mucosal immune system provides a first-line recognition and defence in the airways and other epithelial surfaces. Most T-cells located in the epithelium express the integrin αEβ7 that mediates binding to E-cadherin at the basolateral side of the epithelium [8]. γδ T-cells can mediate specific cellular immune functions without a requirement for antigen processing and can recognize invading pathogens, neoplastic or damaged cells directly [16–18].

In the present study we also found that lymphocytes infiltrate the lung within the first 24 h after BLM treatment (data not shown) and their number increased until Day 4. A fairly stable number of lymphocytes was found until Day 10, with a significant increase thereafter. The CD4/CD8 ratio exceeded 3 in BAL fluid. Similarly, higher numbers of CD4+ cells than CD8+ cells have been found by other groups [19, 20], although this is in contrast with the finding reported by JANICK-BUCKNER *et al*. [21], who reported a higher number of CD8+ than CD4+ cells in the BAL fluid at the initial phase of lymphocyte infiltration. It is difficult to explain this discrepancy. However, it is possible that this disagreement may be due to differences in the dose of BLM (2.7 mg·kg^{-1}) or to a difference in the species. THRALL *et al*. [19] and NETTELBLADT *et al*. [20] used rats, whereas JANICK-BUCKNER *et al*. [21] used C57BL/6 mice, and we used Swiss Webster mice. Both mouse strains are high responders to BLM-induced pulmonary fibrosis and, therefore, a difference in the fibrogenic effect of BLM is unlikely to account for this discrepancy. Further experiments might clarify these differences.

The role of CD4+ or CD8+ T-cells in the regulation of pulmonary fibrosis has been investigated in several models of fibrosis [22–24]. In a mouse model of hypersensitivity pneumonitis, elimination of CD4+ or CD8+ had very little effect on the fibrosis; however, elimination of all Thy1+ T-cells showed a detrimental role for

T-cells in the fibrotic process in the early phase but a beneficial one in the later phase [22]. However, elimination of CD4+ or CD8+ T-cells in a hapten-immune model of pulmonary fibrosis reduced the extent of the fibrosis [23]. In this model, the inflammatory and fibrotic responses could be adoptively transferred with immune lymphocytes, suggesting a regulatory function in the development of the disease. The antigen specific response involves specific CD4+ or CD8+ T-cells and their elimination abrogates the response. In contrast, in bleomycin-induced pulmonary fibrosis, it has been shown that elimination of CD4+ or CD8+ T-cells did not reduce the acute or chronic injury in mice lungs [24], suggesting a non-antigen driven response to the lung injury.

We found that in the T-lymphocyte population a substantial number of γδ T-cells and some NK cells infiltrate the lung after BLM treatment. In earlier reports, the CD4-/CD8-cells were postulated to be NK cells [24] but they might, in part, be γδ T-cells. The function of γδ T-cells in the development of lung fibrosis is unclear, although the evidence available favours a role in regulating the response to BLM-induced tissue damage. It has been reported that γδ T-cells can respond to tumour antigens or microbial antigens directly or in association with heat shock proteins (HSP), or to HSP induced by stress, and recognize damaged tissue [25]. Moreover, γδ T-cells have been shown to establish a primary immune response to pathogens and several lines of evidence have suggested a "first-line of defence" protective role for these cells [26]. BLM induces the HSP 70 promoter and the presence of HSP in highly stressed lungs might provide a conducive environment for γδ T-cells [27].

Recruitment of γδ T-lymphocytes to the lung in response to the presence of HSP derived from alveolar macrophages or damaged epithelium may contribute to the fibrosing response seen in several lung diseases. This hypothesis is supported by experiments in collagen-induced arthritis, where elimination of γδ T-cells prior to the induction of the disease reduced the extent of arthritis [28]. Furthermore, γδ T-cells are capable of secreting a variety of cytokines after stimulation and the presence of γδ T-cells in response to stress in the lung may, therefore, contribute to the priming of the lung to fibrotic processes [26, 29].

In contrast to the pathogenic role of γδ T-cells described in collagen-induced arthritis [28], and a possible role in bleomycin-induced lung fibrosis, γδ T-cells may be part of a circuit involving αβ and γδ T-cells and macrophages [18]. BAL γδ T-cells were shown to be a rich source of the immunoregulatory cytokines interleukin-4 (IL-4), interleukin-10 (IL-10), and interferon-γ (IFN-γ), which are known to promote B-cell growth and antibody synthesis [30], to downregulate the activity of antigen-specific αβ T-cells [31], and to promote or maintain macrophage activation. Results from animal models of viral infections suggest that γδ T-cells, together with αβ T-cells and macrophages, regulate the initiation, progression and, perhaps, resolution of the viral response. In this perception, these cells have a proinflammatory and regulatory role. However, the role of γδ T-cells in the regulation of pulmonary fibrosis in response to injury has so far not been investigated.

γδ T-cells in lymphoid tissues and intestinal epithelium have been reported to express the integrin αEβ7 [32]. Our results show that, in addition, most γδ T-cells found in BAL fluid express the integrin αEβ7. This integrin mediates lymphocyte-epithelial cell adhesion and epithelial homing [33]. The localization in the epithelium supports the idea that the γδ T-cells are involved in establishing the "first-line of defence" protective proinflammatory or regulatory role described. It was observed that the antibodies 2E7 and M290 against αEβ7 act as a co-stimulatory signal with anti-TCR antibodies to increase the lytic machinery of intraepithelial or CD8+ T-cells [34].

This finding is confirmed by the experiments of SARNACKI *et al*. [35], describing a strong synergistic effect of CD3-induced activation and αEβ7 cross-linking on the proliferative response of intraepithelial lymphocytes (IEL). Furthermore, the cytokine profile and ultrastructure of IEL γδ T-cells were found to resemble a cytotoxic effector function [29]. However, not all γδ T-cells reactive with virus-infected cells were found to be cytotoxic [18]; hence, there may be populations of γδ T-cells serving a different purpose. Intraepithelial localization mediated by αEβ7-epithelial cell interaction may prime or activate T-cells for their protective role in this environment. The intensity of activation of IEL may depend on the level of expression of this integrin and we and others have found that the γδ T-cells exhibit a much higher expression of αEβ7 than CD4+ and CD8+cells [32]. These cells may, therefore, show high cytolytic or cytokine secreting activity, a hypothesis not tested in the present study. The possible involvement of integrins in T-cell activation has also been described for other receptors of this family. *In vitro* studies have shown that the lymphocyte-associated integrins, LFA-1 and very late activation antigen- (VLA-4), -5 and -6, on binding to their respective ligands, provide accessory signals which have a synergistic effect on CD3-induced activation of resting peripheral CD4+ T-cells [36]. It is, therefore, possible that the availability of particular integrin ligands in the microenvironment may have a selective influence on the activation of lymphocyte subsets. The high expression of αEβ7 on BAL γδ T-cells and CD4+ T-cells at Days 22 and 30 may preferentially activate these cells and contribute to the development of fibrosis.

In summary, T-lymphocytes of the CD4+, CD8+, NK cell, and γδ T-cell subpopulation migrate into the airways after intratracheal BLM instillation. Within these cells, γδ T-cells have an immunoregulatory role in the early response to pathogens, as well as to tissue damage. Their important location in the epithelium is mediated, at least in part, by expression of the integrin αEβ7 that is different from that of CD4+ and CD8+ T-cells with regard to receptor density and relative amount of cells expressing this integrin. The precise role of these γδ T-cells in the development of pulmonary fibrosis after acute lung injury is not clear but seems to be distinct from allergen-induced fibrosis in hypersensitivity pneumonitis models, where mainly αβ T-cells are mediating the response. The high number of γδ T-cells in the BLM-treated lungs suggests that these cells are an important part in the sequence of events that results in pulmonary fibrosis.

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