Clinical utility of bronchoalveolar lavage cell phenotype analyses in the postoperative monitoring of lung transplant recipients

Martine Reynaud-Gaubert a,b,*, Pascal Thomas a,c, Régine Gregoire d, Monique Badier c, Pierre Cau e, José Sampol e, Roger Giudicelli a, Pierre Fuentes a

a Department of Thoracic Surgery, Sainte Marguerite Hospital, BP 29, 13274 Marseille Cedex 9, France
b Department of Respiratory Medicine UPRES 3287, Sainte Marguerite Hospital, BP 29, 13274 Marseille Cedex 9, France
c Department of Physiology Research, EA 2201, 13916 Marseille Cedex 20, France
d Department of Biostatistics, Sainte Marguerite Hospital, BP 29, 13274 Marseille Cedex 9, France
e Cell Biology and Immunology, Conception Hospital, Marseille, France

Received 29 December 2000; received in revised form 20 August 2001; accepted 18 October 2001

Abstract

Objective: Bronchoalveolar lavage (BAL) fluid provides a crucial tool for investigation of the cellular component of the deep lung spaces and hence to approach the alloreactive response following lung transplantation. This study investigated whether BAL cell profiles can assist for the diagnosis of certain postoperative complications.

Methods: We conducted a retrospective analysis of both transbronchial biopsy and bronchoalveolar lavage materials in a series of 26 consecutive lung transplant recipients (LTR) in relationship with their clinical status at the time of the procedure. BAL fluid was subjected to cell morphology as well as flow cytometric phenotypic analyses. The samples were labeled as follows: normal transplant in clinically stable and healthy recipients, n = 58; acute rejection (AR), n = 58; infection (INF), n = 31; and obliterative bronchiolitis/bronchiolitis obliterans syndrome (OB/BOS) n = 27. Results: Total BAL cell counts were the highest in INF. Lymphocytic alveolitis was suggestive of both acute allograft rejection and CMV viral infection, with a combined significant increased HLA-DR positive cells in AR. Alveolar neutrophilia with an increased CD4/CD8 ratio was correlated with the diagnosis of OB. The neutrophil percentages, HLA-DR and CD57 positive cells were significantly higher when an infection was present. Conclusion: These findings suggest that BAL cell analysis could give complementary information of histological data and further insight into immunologic events after lung allograft. A longitudinal surveillance of BAL cell profiles in an individual patient may be suggestive for a preclinical state of posttransplant acute rejection, bacterial infection and obliterative bronchiolitis. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Bronchoalveolar lavage; Cellularity; Flow cytometry; Lymphocyte-subsets; Lung transplantation

1. Introduction

Bronchoalveolar lavage (BAL) is recognized as the more reliable, accurate and safe method for the routine microbiological detection and diagnosis of infectious lung diseases. Furthermore, it provides a unique tool to evaluate the cellular populations of the lower respiratory tract. Whilst lymphocytes constitute a minor component of BAL cells as compared to alveolar macrophages, their pivotal role in the inflammatory and alloreactive response, has led to focus on this subset. Morphologic and phenotypological analyses of BAL cells have promoted a major interest in the assessment of the degree of activity and in the prediction of the subsequent response to therapy in various lung parenchymal inflammatory processes.

In the field of lung transplantation, histologic examination of lung tissue by transbronchial biopsy (TBB) remains as the gold standard diagnostic procedure for assessing acute allograft rejection. While it is still practiced by many lung transplant programs, the use of surveillance bronchoscopies is getting more and more controversial. Therefore, owing to the invasiveness of this procedure, most lung transplant physicians reserve the use of TBB in symptomatic patients and advocate surveillance biopsies to screen for the occurrence of asymptomatic acute complications only by the first 4 months after transplantation [1]. Furthermore, TBB doesn’t get enough sensitivity in chronic rejection, due to small numbers and/or small size biopsies, the patchy distribution of lesions within the lung, and the high variability of useful bronchial material from TBB [2]. Thus, there may be significant deterioration in function before obtaining a histopathological biopsy findings of
obliterative bronchiolitis (OB). Finally, it is not proven that the practice of surveillance lung biopsies impacts on patient management, and/or improve outcome in terms of survival or freedom from OB [3,4]. Consequently, BAL appeared to be an attractive, reproducible and a less invasive method than TBB to study graft-infiltrating cells.

It has suggested that phenotypic and functional analyses of BAL cells might provide some grounds for knowledge of immunologic status of the allograft [5,6]. We hypothesized that BAL cell morphologic analysis combined with lymphocyte subsets study could offer useful insights into airway immunopathology at a preclinical stage beyond what can be demonstrated with TBB alone. Given previous reports are contradictory, we have conducted a retrospective study to evaluate the usefulness of potential BAL cellular changes as an adjunct to existing sampling procedures used at surveillance bronchoscopy. We report our findings on BAL differential cell counts and lymphocyte subsets determined by flow-cytometry in 26 consecutive lung transplant recipients who underwent concurrent BAL and TBB specimens as a routine part of their clinical evaluation.

2. Patients and methods

2.1. Transplant population

Twenty-six consecutive lung transplant recipients (LTR) (14 males and 12 females) who underwent the transplant procedure between November 1993 and August 1996 and who survived at least 18 months postoperatively were studied retrospectively. The patients’ age at the time of the allograft ranged from 16 to 48 years (mean 29.4 years). The surgical procedures consisted of combined heart-lung (n = 3), double lung (n = 21) or single lung (n = 2) transplantations performed for advanced cystic fibrosis (n = 17), bronchectasis (n = 2), emphysema (n = 2), primary pulmonary hypertension (n = 2), idiopathic pulmonary fibrosis (n = 2) and primary obliterative bronchiolitis (n = 1).

Patients received a standard triple drug immunosuppressive regimen, consisting of cyclosporine, adjusted to maintained whole blood trough levels between 250 and 300 ng/ml, azathioprine (1 mg/kg per day) adjusted to white blood cell count (above 4000/mm³), and oral steroids tapered to 0.25 mg/kg per day over the first 3 months and stopped by the sixth postoperative month if lung function remained stable. Rabbit antithymocyte globulins (Pasteur Merieux, France) were given for the first 3 days. Episodes of symptomatic acute cellular allograft rejection were treated with bolus intravenous methylprednisolone (10 mg/kg per day on 3 consecutive days). Cytomegalovirus (CMV) prophylaxis consisted of IV ganciclovir (5 mg/kg twice daily) for 21 days followed by a 10-week course of oral acyclovir (2400 mg/d). Recipients who were CMV-seronegative receiving organs from CMV-seronegative donors did not receive any CMV prophylactic therapy. CMV pneumonitis was treated by a minimal 4-week course of IV ganciclovir.

2.2. Postoperative follow-up

Patients underwent regular follow-up visits at the hospital for clinical and radiological evaluation. Pulmonary function tests (PFTs) were measured monthly within the first 12 postoperative months and every 2–3 month thereafter.

During this study period, patients underwent bronchoscopy with concomitant BAL and TBB specimens were performed as part of a prospective surveillance protocol at approximately 1, 3, 6, 9, 12, 18 and 24 months after surgery. Additional bronchoscopies were performed after specific treatment of acute complications, and occasionally for clinical indications. Samples were taken from after sedation through a flexible fiberoptic bronchoscope wedged into a subsegmental bronchus of either the lingula or the right-middle-lobe. BAL was done by instilling a total volume of 250 ml of sterile isotonic saline solution warmed up at room temperature in sequential 50-ml aliquots. The fluid fractions were aspirated with a gentle low-pressure suction into a siliconized glass container. The retrieved fluid was pooled, mixed, and the total recovered volume was measured. Samples of fluid decanted into polypropylene flasks were processed immediately for cytological, microbiological and immunological analyses. TBB specimens were obtained immediately afterwards from the lung periphery under radioscopic control, using an alligator forceps. Three or four biopsies were obtained from each lobe of one lung.

2.3. Laboratory methods: analysis of BAL cells

2.3.1. Total and differential cell counts

Red blood cells and nucleated cells from fresh, uncultivated neft BAL were counted using a Kova Slide (Boehringer Mannheim, Germany). A total of 100 μl aliquots of BAL fluid were cytocentrifuged (700 rev./min/ for 2 min) and spotted on silane-coated slides (Sigma Chemical Co) using a Cytospin II (Shandon Southern Instruments, Sewickley, PA, USA) in order to identify the nucleated cells. Cytospin preparations were stained by using May Grunwald Giemsa, the two others using Papanicolaou and Perls method to detect ferric pigments. Results were expressed as percentages of total BAL cell counts. All samples were analyzed by the same trained cytology assistant in a blinder manner.

2.3.2. Flow cytometric (FC) phenotypic analysis

The aliquots of BAL fluid were centrifuged (500 × g for 10 min) to sediment the cells which were then washed twice in phosphate buffered saline containing bovine serum albumin (PBS-B-A) 1% and sodium aide. The cell suspension was adjusted to a final cell count of 1.10⁷ cells/ml. The cell suspensions were incubated with a panel of monoclonal antibodies (mAb) listed in Table 1. The staining panel for lymphocytes typing included anti-CD3,
-CD4, -CD8, -HLA-DR, CD37 and CD57. The second reagent for indirect immunofluorescence assay was a goat polyclonal anti-mouse Ig (Silenus, Eurobio, Paris, France). An indirect immunofluorescent assay (IIFA) was performed using a standard protocol: aliquots (100 μl) of cell suspension (10⁶ cells) were incubated in individual test tubes with 10 μl of each mAb for 20 min at 4°C, then washed twice in PBS-B-A. A total of 100 μl (1×10⁶ cells) of the second step reagent were added at a dilution of 1/300 in PBS-B-A for 20 min at 4°C. For samples contaminated by peripheral blood, hemolysis was performed with a solution of ammonium chloride. After two additional washes, cells were resuspended in PBS-B-A and analyzed in flow cytometry. This analysis was performed on a Coulter Epic XL flow cytometer equipped with four decades logarithmic amplifiers (Coultronics, Margency, France). Instrument optical alignment, laser output and photomultiplier settings were optimized daily, using uniform polystyrene particles containing fluorescent dyes (DNA-Check, Coultronics, Margency, France). Lymphocytes were identified on the basis of double scatter, and files were gated to include only cells within the window set of these parameters. Gating was adjusted and purity assessed using appropriate controls ensuring the selection of more than 90% of CD45 positive leukocytes. Data for 5000 lymphocytes were collected and results expressed as percentage of fluorescent cells for each marker.

2.4. Data analysis

Serial BAL procedures performed on each subject were considered as separate events if separated from at least 1 month. Each of the bronchoscopy procedures (BAL and concurrent TBB) was allocated to one the four groups according to clinical, microbiological and histopathological status at the time of the procedure as outlined below.

2.4.1. Group 1

Clinically stable LTR (17 patients) with neither rejection nor infection and normal histologic features at routine surveillance TBB. These specimens were considered as controls (n = 58).

2.4.2. Group 2

Acute rejection (AR), n = 58 occasions (14 patients), of which 43 were classified as of minimal (A1), 12 mild (A2) and three moderate (A3) character. Rejection was graded according to the International Society for Heart and Lung Transplantation (ISHLT) criteria [7].

2.4.3. Group 3

Obliterative bronchiolitis (OB/BOS): n = 27 occasions (ten patients), 18 of which have been biopsy-proven OB upon the standardized nomenclature [7]. The diagnosis of bronchiolitis obliterans syndrome (BOS) was established according the spirometric criterion (the decline in FEV₁) proposed by the ISHLT [8]. Because of possible the confounding influence of airway infection on the measured makers, we excluded infected BOS patients from this group. Only BOS patients clinically free of infection, who were afebrile, with no recent change in physical signs, peripheral blood leukocyte count, chest radiograph or bronchoscopic examination were analyzed in this group (n = 21).

2.4.4. Group 4

Infection alone (INF), n = 31 specimens obtained from 15 patients using histopathological data and cultures from BAL fluid. This group included bacterial (n = 19), fungal (n = 2), and CMV infections (n = 10).

Specimens (n = 62) were excluded from analysis when (1) they were obtained during the first 2 months after surgery; and (2) there was an overlapping diagnosis (e.g. both lung infection and acute rejection) because they could not be assigned to any single diagnostic group.

2.4.5. Statistical analysis

Data were analyzed by using the SPSS/PC software package (SPSS Inc., Chicago, IL). Values are expressed as means ± SEM. Additionally, in order to improve the potential clinical relevance of BAL cell phenotype abnormalities, we determined the significant value for a change from the confidence interval (CI) that was calculated for each studied variable, using the measurements obtained in the control group. Groups were compared for significant differences using the non-parametric Mann–Whitney U-test. Any P value of 0.05 or less was considered as statistically significant.
3. Results

The median time of follow-up for the cohort was 28 ± 17 months (range 18–69 months) during which a total of 174 representative TBB specimens and BAL fluid samples were performed and selected for analysis (median 6.7 bronchoscopies per patient; range 6–10).

3.1. Cell counts and proportions

There were no significant differences in total volumes of retrieved BAL fluid in the different diagnosis groups. Results of total BAL nucleated cells and differentials are summarized in Table 2. Total cellularity was significantly higher in INF, in AR and in OB/BOS groups) than in controls (P < 0.01, respectively).

There was a decreased percentage of alveolar macrophages in the three pathological entites (P = 0.0001, respectively), but especially in INF and OB populations (OB and INF vs AR, P < 0.01, respectively) when compared to the healthy patients.

Compared to controls, the highest BAL lymphocyte counts were found in AR (P = 0.0001), and at a lowest level in INF. The relative proportion of lymphocytes was significantly lower in OB than in clinically well patients. Alveolar lymphocytosis was significantly different between the four diagnosis groups, except for the comparison between controls versus INF. There was no relationship between BAL lymphocyte percentages and the degree of acute rejection. A signifcant change in alveolar lymphocytosis outside the CI (>20% of the total BAL leukocytes), that was defined by using the measurements from controls and IN groups, was highly suggestive of acute rejection. Sensitivity, specificity, positive and negative predicted values of BAL lymphocytosis for the diagnosis of AR were 65, 92.5, 79.5 and 85.4%, respectively. In 14 specimens included in the histological AR group, lymphocytosis was still in the CI. Conversely, in five of 44 specimens (11%) with a significant increase in lymphocyte percentages, the corresponding clinical diagnosis was an infection, mainly a CMV pneumonia (four of the five BAL specimens).

The highest relative BAL neutrophil counts were found in OB and infected patients (P < 0.0001 for both), and there were differences between the three pathologic groups. The decreasing order of neutrophil counts was OB, followed by INF, and AR. A significant change in alveolar neutrophilia outside the CI (>43% of the total BAL leukocytes), that was defined by using the measurements from controls and AR groups, was significantly associated with OB (P < 0.01), with 82% sensitivity, 94% specificity, 71 and 94.5% positive and negative predicted values respectively. A significant change in neutrophil percentage was detected on 31 occasions, 22 of which were associated with an OB diagnosis whereas it appeared in only nine cases out of 147 occasions without OB. In these nine cases, the corresponding clinical diagnosis was an acute rejections (n = 2), or a bacterial infection (n = 7).

BAL eosinophilia above 5% of the total nucleated cells was encountered in 12 instances overall (three patients). Increased numbers of eosinophil were associated with an acute rejection in nine out of 12 instances (seven were classified as of grade A1 and two of grade A2), with a CMV infection in one case and a TBB considered as normal in the remaining two cases.

3.2. Flow cytometry

Flow cytometry analysis of lymphocytes in BAL showed phenotypic differences according to the clinical status (Table 3). The proportions of CD3+ T cells were not significantly different in the 4 diagnosis groups (data not shown). Regarding the distribution of the CD4 and CD8+ lymphocytes subsets, the OB group expressed significant increased percentage of CD4+ and decreased numbers of CD8+ cells in BAL samples, with a significantly increased CD4/CD8 ratio, as compared with controls, AR and the infected group samples (P < 0.001). During infections, and especially in CMV pneumonias, the CD4/CD8 ratio decreased due to lowered percentages of CD4+ cells in BAL (P < 0.05). There were no significant differences in CD4 and CD8+ lymphocyte expression between healthy recipients and rejecting patients.

Concerning the expression of the activation marker of HLA antigen, the proportion of HLA-DR+lymphocytes was higher during infection and acute rejection (P = 0.001 for both) than in controls. There was no statistically significant difference between the other groups for the HLA-DR marker. The expression of natural killer cell associated surface antigen CD57 was consistently higher in infected patients as compared with the three other group (P < 0.05, respectively). The CD37 marker of lymphocytes

Table 2
Total and differential white cell counts in BAL from lung transplant recipients

<table>
<thead>
<tr>
<th>Clinical subgroups</th>
<th>Normal (n = 58)</th>
<th>Acute rejection (n = 58)</th>
<th>Infection (n = 31)</th>
<th>OB (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell counts (× 10³/ml)</td>
<td>326 ± 34</td>
<td>641 ± 81**</td>
<td>1351 ± 428*</td>
<td>717 ± 175</td>
</tr>
<tr>
<td>Macrophages (%)</td>
<td>81 ± 2</td>
<td>54.6 ± 3.2***</td>
<td>40.2 ± 4.8***</td>
<td>22.2 ± 3.3***</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>10.3 ± 1.6</td>
<td>28.7 ± 2.5***</td>
<td>18.4 ± 4.3</td>
<td>5.9 ± 1**</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>7 ± 1.5</td>
<td>12.3 ± 2.3</td>
<td>38.8 ± 6***</td>
<td>68 ± 4***</td>
</tr>
</tbody>
</table>

*a P < 0.05, **P < 0.001, ***P < 0.0001 versus normal, respectively.
showed poor immunostaining (mean below 4%), and did not permit comparison between the four groups.

4. Discussion

Morphologic analysis of BAL cells in our lung transplant recipients revealed that (1) in samples from the control group, the cellularity was higher than in healthy nonsmoking volunteers [9], with increased proportions of neutrophils and lower percentages of macrophages; and (2) BAL cellularity may be suggestive for certain posttransplant complications. The proportion of BAL lymphocytes was significantly increased in patients with acute rejection. The relative very small number of moderate acute rejection (three grade A3 only) did not permit to find BAL cell changes between the different degrees of acute rejection. Neutrophils were increased in patients with infection and so especially in subjects who experienced post-transplant OB. A sensitivity analysis for specific selected threshold values of lymphocytes and neutrophils in BAL emphasizes that BAL cell differentials may be helpful in the diagnosis of complications in lung transplant recipients. In this setting, previous published results are contradictory. Bronchoalveolar lavage profiles have been looked at in many studies and have proven to be neither reproducible nor to be sensitive and specific enough to be relied on for accurate diagnosis [10,11]. It is likely the result of the influence of different methods of retrieving lavage and dealing with it might account for differences in absolute numbers of cells retrieved. Moreover, we met difficulties with the definition of large homogeneous diagnosis groups. The study presented highlights the problems with diagnosis in transplantation, in that on many occasions dual pathologies exist and these implicitly require histological confirmation of diagnosis [12]. We had to deal with the possible confounding effect of infection. From a practical point of view, this is a common problem faced by many of BAL studies in human lung transplantation as both infection and other clinical status (i.e. OB) may be present in a similar fashion [12,13]. We can think that such approach contributed to a lack of specificity of our results in the infected group. In agreement with the results of previous studies [10], we observed increased lymphocyte percentages in BAL in the presence of acute rejection. Similarly, in accordance with other investigators [10,13–17], our data showed BOS to be closely linked with BAL neutrophilia. Currently, it is still difficult to define the exact contribution of neutrophil activation in BAL to the pathogenesis of OB. Some recent studies reported a parallel increase in alveolar neutrophilia and the secretion of neutrophil chemoattractant chemokine interleukin-8 (IL-8) [13], eosinophil cationic protein and myeloperoxidase, used as markers for the activation of neutrophils [14] in BOS patients. More recent works underlined that airway neutrophilia constituted an early indicator of OB [13,16] and persisted despite enhanced immunosuppression in patient with a subsequent poor survival [17].

In our study three patients (12 BAL samples) presented with severe recurrent episodes of eosinophilic alveolitis in BAL. In nine cases, BAL eosinophilia was associated with acute rejection. Other authors have observed similar data in a small number of patients, who exhibited an increased percentage of eosinophil in BAL during acute rejection [18,19], CMV disease or bacterial infection [20]. Eosinophils might be activated in rejecting allograft and release cytotoxic eosinophil cationic protein [20]. It was hypothesized that the eosinophil could be triggered by release of cytokines from both activated helper T-lymphocytes and antigen-presenting macrophages in the allograft [18].

Lymphocyte subtyping in BAL by flow cytometry using a DNA-dye, which offers a rapid and reliable method [21]. This analysis method has been widely used to differentiate various immune-mediated lung disorders characterized by a lymphocytic alveolitis, such as sarcoidosis from other causes of interstitial pulmonary fibrosis. The focus prior research has been on lymphocyte count and subsets, especially CD4 and C8 positive cells. Regarding the distribution of the CD4 and CD8+ lymphocytes subsets, the clinically stable LTR displayed lower percentages of CD4+ and higher proportions of CD8+ cells in BAL samples, as compared with healthy nonsmoking volunteers [9], resulting in a reduced CD4/CD8 ratio (0.6 versus 1.9, respectively).

In human pathology, patients with pulmonary sarcoidosis frequently have increased percentage of lymphocytes, with a high CD4/CD8 ratio in BAL. However, it has been shown more recently that BAL CD4/CD8 ratio measured during the initial diagnostic evaluation of patients with sarcoidosis was highly variable and that BAL lymphocyte subset determination would have a low diagnostic sensitivity for this disease [22], at least at the early phase of disease progres-

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Monoclonal antibody expression (%) on BAL lymphocytes from LTR¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical subgroups</td>
<td>Normal (n = 33)</td>
</tr>
<tr>
<td>CD4 (%)</td>
<td>21.2 ± 2.2</td>
</tr>
<tr>
<td>CD8 (%)</td>
<td>41 ± 2.8</td>
</tr>
<tr>
<td>CD4/CD8</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>HLA-DR (%)</td>
<td>19.6 ± 2.8</td>
</tr>
<tr>
<td>CD57 (%)</td>
<td>15.2 ± 12.1</td>
</tr>
</tbody>
</table>

* P < 0.05, **P < 0.001, ***P < 0.0001 versus normal, respectively.
sion. In the field of lung transplantation, phenotypic analysis of lymphocytes gives contradictory results. In the present study, we did not find significant difference in CD4/CD8 ratio in BAL between stable LTR and the acute rejection group. Previous studies reported that CD8+ lymphocytes were increased in BAL with a trend for a lower mean CD4/CD8 ratio in acute lung rejection when compared to the group with no evidence of AR [5,23,24]. We found that patients affected with OB/BOS had an increased percentage of CD4+ cells and decreased CD8+ lymphocytes, resulting in a significantly higher BAL CD4/CD8 ratio, consistent with the finding of Paradis I et al. [25]. Other investigators demonstrated that BOS as well as acute rejection was frequently associated with a decreased CD4/CD8 ratio compared with healthy recipients [5,12,24]. As observed in many studies, we found a CD8+ over-expression with a marginally lower CD4/CD8 ratio in infected recipients, particularly during CMV infections, than when infection was absent [5,24]. The discrepancies between the different studies could depend upon several factors. Firstly, the difficulties encountered in the assessment of lymphocyte subsets in BAL samples, with a relatively low number of lymphocytes retrieved in BAL fluid during bacterial infections and particularly during OB. In our study, only two/three of BAL samples performed during infection and one/three of BAL associated with OB/BOS could be available for lymphocyte analysis by flow cytometry. Secondly, the frequency with which the BAL subset analysis is performed should be evaluated. BAL cell profiles should be highly variable depending on the stage of disease progression, especially in OB with a prolonged evolution, and the transplant center surveillance protocol.

The HLA-DR and CD57+ lymphocyte markers were also found as clinically relevant. With regard to the expression of HLA-DR on BAL cells, there is a great variation in healthy volunteers subjects [9,26]. Deduced by the HLA-DR mAb marker use, we observed an enhanced expression of class II MHC antigens by BAL cells during acute rejection and moreover when infection was present. The association between the presence of activated lymphocytes bearing HLA-DR antigens and allograft rejection already has been reported [27]. Other investigators shown an enhanced expression of class II antigens on both epithelial and endothelial cells from lungs with lesions of OB [28,29]. The mAb Leu-7, which reacts with the CD57 antigen of natural killer cells, was highly expressed in our infected patients, and so might be an additional marker of this complication. At our knowledge, this marker has not been studied in BAL from lung transplant recipients. Finally, our findings and those from the literature may suggest a local disturbance of cellular immunoregulatory functions in the genesis of postoperative complications. Further collaborative studies would be needed to determine the functional activities of the T-cells populations and to elucidate the mechanism of CD4+/CD8+ imbalance in the lung allograft.

We studied single features of BAL cellular profiles in our sensitivity analysis. It should be potentially more profitable to examine the overall pattern of bronchoscopic data, involving more than one feature in a multivariate analysis in a larger cohort of patients to increase the sensitivity of the BAL markers. Additionally, BAL cellularity could be particularly useful in clinical assessment after single lung transplantation. In this setting, which concerns the majority of patients who undergo transplants around the world, the clinical follow-up is mostly difficult because pulmonary functional parameters cannot be extrapolated to the single transplanted organ when the disease worsens in the native lung. Our experience does not support the routine clinical use of BAL cellular phenotyping to diagnose complication. It suggests a potential interest in the immunological status monitoring of the allograft in individual patients. Thus, an enhanced lymphocytosis and/or eosinophilia together with an overexpression of HLA-DR+ cells and a lower CD4/CD8 ratio in BAL, without evidence of lung infection, could suggest an alloreactive response and could require, even in asymptomatic patients, TBB specimens to look for an acute rejection. Similarly, progressive increased neutrophil percentage without evidence of respiratory infection might be considered as predictive marker for OB. These findings emphasize the clinical relevance of a longitudinal study of BAL cell populations, even in still a stable patient, as a diagnostic adjunct of a negative lung biopsy or at a preclinical stage of posttransplant complication. TBB could be reserved to clinically symptomatic patients or patients who exhibit abnormalities in BAL cell profiles which are not explained by evidence of pulmonary infection or functional diagnosis of BOS.

In conclusion, surveillance BAL cell profiles can assist monitoring of the main postoperative complications. The clinical relevance of BAL cell findings should be confirmed in longitudinal studies because of criticism that surveillance TBB are both invasive and not sufficiently sensitive in OB. Given the limitations of our work, a prospective multicenter study should be useful to look at the value of surveillance BAL in lung transplantation. The present results could provide a platform on which such an investigation would be based. Then, we can hope to avoid delayed or deleterious high level of immunosuppression and to develop a clinical modulation of therapeutic strategies even before histopathological lesions become evident.

Acknowledgements

This study was supported by Assistance Publique-Hôpitaux de Marseille.

References


