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Alveolar macrophages in idiopathic pulmonary fibrosis display a more monocyte-like immunophenotype and an increased release of free oxygen radicals

J. Kiemle-Kallee*, H. Kreipe**, H.J. Radzun**, M.R. Parwaresch**, U. Auerswald***, H. Magnussen***, J. Barth*

Alveolar macrophages in idiopathic pulmonary fibrosis display a more monocyte-like immunophenotype and an increased release of free oxygen radicals . J. Kiemle-Kallee, H. Kreipe, H.J. Radzun, M.R. Parwaresch, U. Auerswald, H. Magnussen, J. Barth.

ABSTRACT: Bronchoalveolar lavage (BAL) was performed in 13 patients with idiopathic pulmonary fibrosis (IPF) and in 10 control subjects. Free oxygen radical (FOR) production of alveolar macrophages (AM) and of blood monocytes was measured by luminol-dependent chemiluminescence (LDCL) without and after stimulation with zymosan A. We confirmed earlier studies that alveolar macrophages of IPF patients display a significantly elevated release of FOR under basic and under stimulated conditions. In contrast to alveolar macrophages, blood monocytes did not reveal altered LDCL in IPF. This indicates that the functional properties for augmented FOR release by IPF alveolar macrophages are acquired in the lungs and not in the peripheral circulation.

To elucidate the possible mechanisms leading to the augmented LDCL response, immunophenotyping of alveolar macrophages was carried out. A panel of new monoclonal antibodies of the Ki-M-series, discriminating differentiation stages of monocyte/macrophage subpopulations, served for immunocytochemical staining. In IPF patients distribution of Ki-M2, Ki-M3, Ki-M6 and Ki-M8 positive AM demonstrated an increased proportion of alveolar macrophages expressing a more monocyte-like immunophenotype. Normally, monocytes as precursors of AM reveal a markedly stronger LDCL than alveolar macrophages themselves. Therefore, it seems likely that the increased LDCL of alveolar macrophages in IPF is due to the higher proportion of more immature monocyte-like cells in the alveoli of these patients.

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* I. Medizinische Klinik and ** Institute of Pathology, University of Kiel, Schittenhelmstr 12/Hospitalstr. 42, 2300 Kiel 1, Germany.

*** Krankenhaus Großhansdorf, Zentrum für Pneumologie und Thoraxchirurgie, Wöhrendamm 80, 2070 Großhansdorf, Germany.

Correspondence: Dr J. Barth, I. Medizinische Klinik, University of Kiel, Schittenhelmstrasse 12, 2300 Kiel 1, Germany.

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In many disorders with pulmonary involvement alveolar macrophages (AM) are claimed to play a major role in the destruction of normal lung architecture [1]. Knowledge about the interactions of respiratory cells leading to fibrosis was especially increased by bronchoalveolar lavage (BAL). In pulmonary fibrosis the predominate change of BAL is the occurrence of neutrophilic and eosinophilic granulocytes at sites of tissue destruction [2]. Neutrophil granulocytes contribute to initiation and maintenance of pulmonary fibrosis [3]. CLARK and KLEBANOFF [4] provided evidence for the involvement of oxidative metabolism in neutrophil cytotoxicity.

The release of free oxygen radicals which had been evaluated in polymorphonuclear granulocytes is influenced by macrophages as well. Investigations of NATHAN and COHN [5] stressed the importance of monocyte/macrophage free oxygen radical production in tissue injuring mechanisms. Reports of CLEMENT *et al.* [6] indicate that decline of oxygen metabolite generation correlates with improvement of clinical, radiological and functional parameters. Based on these results the state of oxidative metabolism of monocyte/macrophage cells in pulmonary fibrosis became of more interest. Recently alveolar macrophages were reported to carry the major burden of free oxygen radical production in idiopathic pulmonary fibrosis (IPF) [7].

It is not yet clear if this property is acquired by monocyte/macrophages while they are in the peripheral circulation or by cells at site of injury in the lungs.

The release of free oxygen radicals correlates strongly with chemiluminescence [8]. Furthermore, luminol-dependent chemiluminescence (LDCL) requires the presence of peroxidase activity [9]. We used the LDCL

technique to investigate the free oxygen radical production by alveolar macrophages and by blood monocytes in IPF.

There is convincing evidence that alveolar macrophages derive from monocytes [10, 11]. During maturation, each stage of differentiation is characterized by a typical pattern of antigen expression that can be shown by monoclonal antibodies of the Ki-M-series [11–14]. We investigated whether differences of functional properties as revealed by luminol-dependent chemiluminescence were associated with changes in the alveolar macrophage stage of differentiation in IPF patients compared to controls. Additionally, possible correlations between free oxygen radical release and HLA-DR and -DQ major histocompatibility complex (MHC) class II antigen expression were analysed.

Materials and methods

Patients

Thirteen patients with idiopathic pulmonary fibrosis (10 men, 3 women, mean age 47.3±5.8 yrs) were studied. None of these patients received immunosuppressive therapy at time of investigation. Diagnosis of lung fibrosis was confirmed by typical radiographic features and histologically by transbronchial or open lung biopsies. Alveolar macrophages from 10 patients with unilateral peripheral bronchial carcinomas served as control group (9 men, 1 woman, mean age 56.1±5.9 yrs). In these latter cases the nonaffected lung, where no clinical, radiographic or histological sign of systemic parenchymal lung disease could be found, was taken for lavage. Blood monocytes were investigated in 5 patients out of each group. All studied subjects were nonsmokers.

Separation of monocyte/macrophage cells

Bronchoalveolar cells were obtained by lavaging the middle lobe in IPF and the middle lobe or the lingula in controls using 5×20 ml physiological saline as described previously [2]. The recovery was between 50–70%.

Separation of BAL cells was performed by centrifugation at 200 g for 10 min followed by washing in Dulbecco's modified Eagle's medium. Mononuclear cells were isolated by Ficoll-Hypaque gradient centrifugation at 800 g for 10 min at 4°C [15]. In a further step alveolar macrophages were separated by adherence to glass culture dishes [16].

Monocytes were isolated from fresh heparinized (40 $IU \cdot ml^{-1}$) venous blood following density gradient centrifugation and glass adherence as described above.

After the separation of alveolar macrophages and of blood monocytes contamination by granulocytes was less than 2% on all slides. Viability confirmed by trypan blue staining exceeded 90%.

Chemiluminescence

Cells were removed from the dishes with a rubber stick and suspended in RPMI 1640 medium (Serva, Heidelberg, Germany). Chemiluminescence assay was performed as described by BARTH *et al.* [17]. The number of cells was adjusted to 2,000 $cells \cdot \mu l^{-1}$. Five hundred µl of the adherent cell suspensions were pipetted into darkness-adapted vials for Berthold biolumate Counter model LB 9505 (Berthold, Wildbad, Germany). To enhance chemiluminescence, 20 µg luminol 2×10^{-7} M (5-amino-2,3-dihydro-phtalazine 1,4-dione obtained from Sigma, Munich, Germany) was added. After preincubation for ten minutes at 37°C monocyte/macrophage cells were stimulated by 1 mg zymosan A from *Saccharomyces cerevisiae* (Sigma, Munich, Germany) suspended in 20 µl Eagle's medium. Data represent the peak of LDCL response which was recorded over 40 min.

Table 1. – Reactivity of Ki-M monoclonal antibodies as revealed by immunohistochemical staining on alveolar macrophages and stimulated blood monocytes in control subjects according to KREIPE *et al.* [12] and RADZUN *et al.* [13]

	Stimulated blood monocytes	Alveolar macrophages
Ki-M2	-	- -
Ki-M3	-	-
Ki-M6	+	++
Ki-M8	+	+

-, +: respectively, less and more intense staining pattern compared to normal blood monocytes.

Monoclonal antibodies

Immunophenotyping of alveolar macrophages was accomplished using monoclonal antibodies of the Ki-M-series (developed at the Institute of Pathology, University of Kiel, Germany, also available from Behring, Marburg, Germany) which can evaluate the differentiation stage of monocyte/macrophage subpopulations (table 1) [11–14]. Two of the applied antibodies, Ki-M2 and Ki-M3, characterize cell membrane antigens the expression of which decreases with maturation of monocytes into macrophages. In contrast, the Ki-M6 and Ki-M8 antigens are localized intracytoplasmatically and show increased density in macrophages compared to monocytes.

Antibodies directed against the (MHC) class II antigens HLA-DR and -DQ were achieved from Becton and Dickinson (Heidelberg, Germany).

Immune alkaline phosphatase technique was applied to the adherent BAL cell fraction using a three-step incubation procedure. After application of the primary mouse antibody, rabbit anti-mouse antisera conjugated with alkaline phosphatase (Sigma, Munich, Germany) was added. To augment the dye development we used a

second monoclonal antibody, goat anti-rabbit IgG, which was also conjugated with alkaline phosphatase (Sigma, Munich, Germany). Ki-M and MHC class II antigens were made visible with Fast Red TR (Sigma, Munich, Germany) for staining alkaline phosphatase as described in detail by FELLER *et al.* [18]. To quantify the reaction, all slides were viewed under a light microscope with a magnification of 100–400. The percentage of positive alveolar macrophages was determined by evaluating 500 adherent BAL cells by two independent observers.

Table 2. – Lung function data (percentage of predicted values) and percentage of cells in the bronchoalveolar lavage of 13 patients with idiopathic pulmonary fibrosis (IPF) and of 10 control subjects

	IPF	Controls
DLCO %	59.6±11.4	98.5±8.9
FVC %	68.2±9.7	92.3±5.7
TLC %	74.5±9.6	94.6±4.8
AM %	78.3±6.0	91.7±3.6
PMN %	7.6±3.4	1.1±0.3
Eos %	2.2±1.3	0.1±0.0
Lymph %	12.3±5.3	7.2±2.7

DLCO: single breath carbon monoxide diffusing capacity; FVC: forced vital capacity; TLC: total lung capacity; AM: alveolar macrophages; PMN: polymorphonuclear leucocytes; Eos: eosinophilic granulocytes; Lymph: lymphocytes. Mean±SD.

Statistical analysis

If not noted otherwise data were expressed as median with standard error of median (SEMd). Comparisons between groups were made with the U-test (Mann-Whitney-Wilcoxon) [19].

Results

Lung function data and the results from BAL cytology are presented in table 2.

Chemiluminescence of alveolar macrophages and blood monocytes

Alveolar macrophages of all studied subjects revealed a basic luminol-dependent chemiluminescence considerably lower than zymosan stimulated LDCL.

Unstimulated alveolar macrophages of IPF patients showed significantly greater LDCL than AM from the controls (fig. 1). Stimulation by zymosan resulted in an oxidative burst, visible as a five to sixfold enhancement of chemiluminescence in alveolar macrophages during the phagocytosis of zymosan, both in the control group and in IPF patients. Consequently we also found a significantly higher level of LDCL in stimulated alveolar macrophages of patients with idiopathic pulmonary fibrosis compared to the controls.

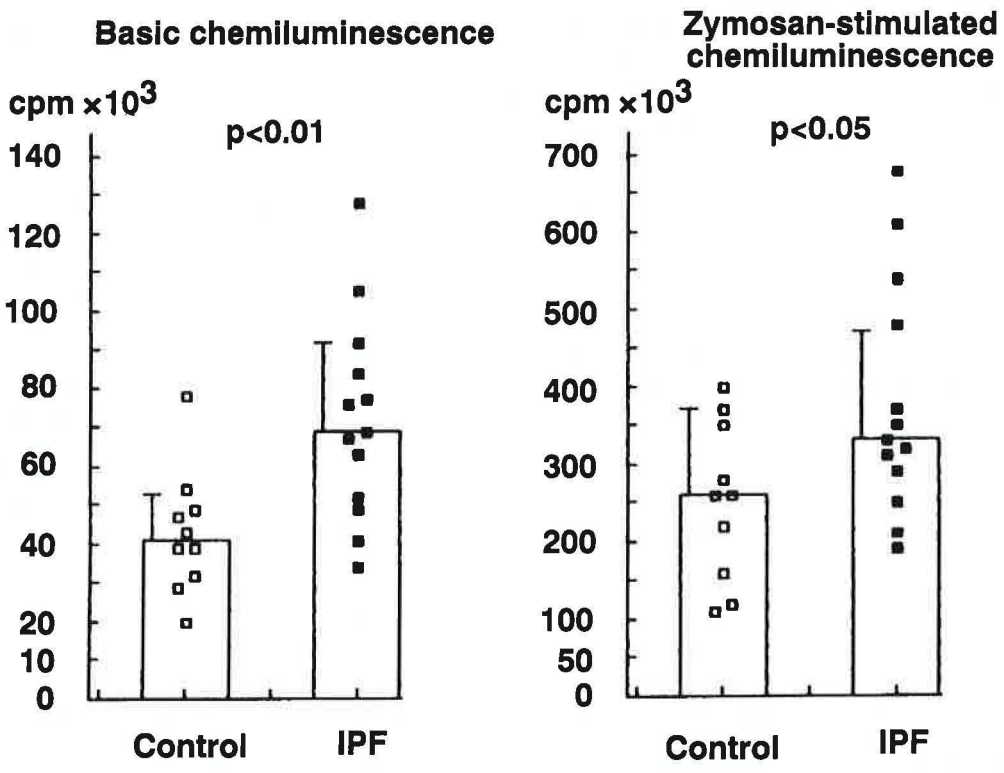

Fig. 1. – Basic and zymosan-stimulated luminol-dependent chemiluminescence released by alveolar macrophages from patients with idiopathic pulmonary fibrosis (IPF, n=13) and control subjects (n=10). Peak counts per minute (cpm) of 2,000 cells·μl^{-1}, 2×10^{-7} M luminol. Median±SEMd. U-test.

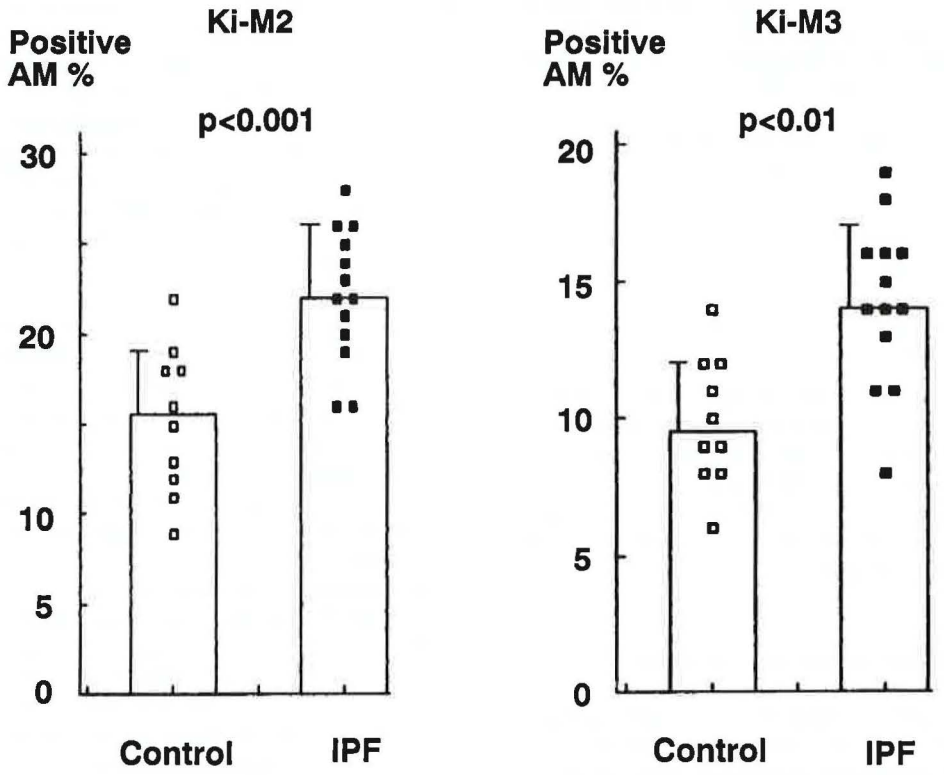

Fig. 2. – Percentage (%) of alveoler macrophages (AM) from patients with idiopathic pulmonary fibrosis (IPF, n=13) and from control subjects (n=10) immunoreacting with monoclonal antibodies, Ki-M2 and Ki-M3, the corresponding antigen expression of which decreases during maturation of monocyte/macrophage cells. Median±SEMd. U-test.

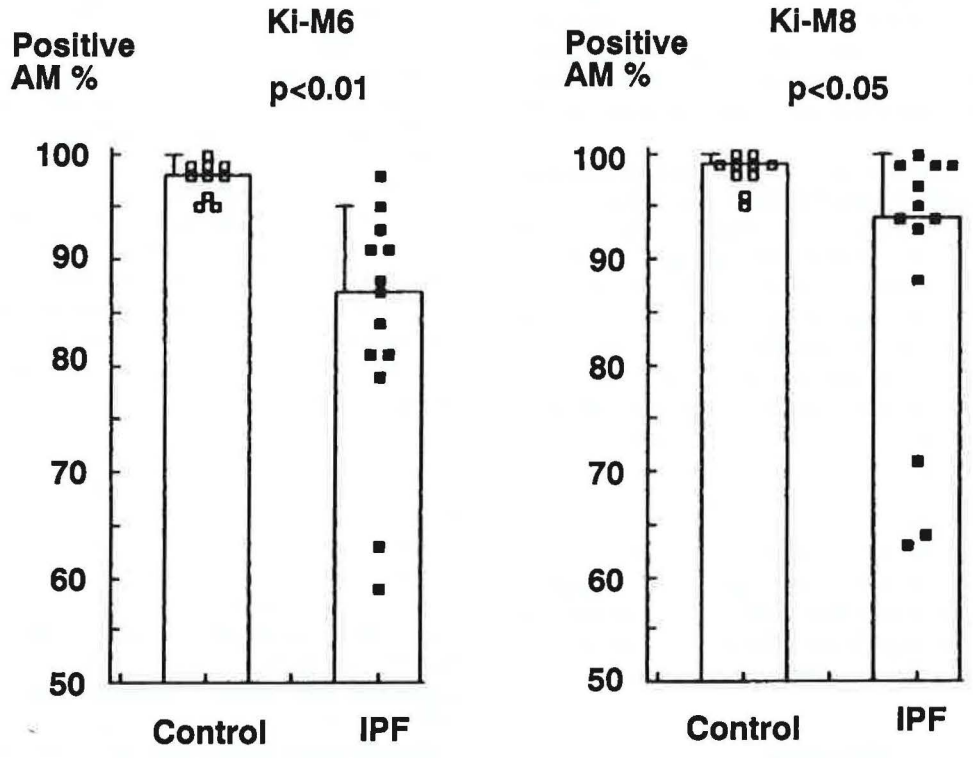

Fig. 3. – Percentage (%) of alveolar macrophages (AM) from patients with idiopathic pulmonary fibrosis (IPF, n=13) and from control subjects (n=10) immunoreacting with monoclonal antibodies, Ki-M6 and Ki-M8, whose corresponding antigen expression increases during maturation of monocyte/macrophage cells. Median±SEMd. U-test.

Table 3. – HLA-DR and -DQ major histocompatibility complex (MHC) class II antigens expressed by alveolar macrophages of patients with idiopathic pulmonary fibrosis (n=13) compared to controls (n=10)

MHC class II antigen	Percentage of positive alveolar macrophages		U-test
	IPF patients	Controls	
HLA-DR	93±3 (89–99)	92.5±3.5 (87–99)	NS
HLA-DQ	89±5 (85–98)	95.5±3.5 (89–99)	NS

IPF: idiopathic pulmonary fibrosis; NS: not significant. Values are median±SEMd (range).

In contrast to alveolar macrophages, comparison of blood monocytes from IPF patients and controls revealed no significant difference of LDCL response. This result was observed under basic (IPF 4.05±0.84, controls 4.13±0.79 counts per minute $\times 10^5$; $p>0.1$) and under stimulated conditions (IPF 4.14±1.01, controls 4.21±0.97 counts per minute $\times 10^6$; $p>0.1$).

Reactivity pattern with monoclonal antibodies

There was less than one third of alveolar macrophages staining with Ki-M2 or Ki-M3 monoclonal antibody in IPF and in controls. Comparison between groups revealed a significantly stronger immunoreactivity of Ki-M2 and Ki-M3 in patients with idiopathic pulmonary fibrosis (fig. 2).

Staining with Ki-M6 showed a strong response with at least 95% of positive alveolar macrophages in control subjects. In IPF patients reactivity of Ki-M6 was significantly reduced but still high. Similar results to the latter antibody were found concerning Ki-M8, which displayed at least 95% of immunoreactive AM in controls and a significant reduction on a still high level in IPF patients (fig. 3).

The different immunoreactivity expressed in quantitative terms by counting stained alveolar macrophages could be confirmed by qualitative investigation. Ki-M2 and Ki-M3 revealed a less, and Ki-M6 and Ki-M8 a more, intense staining pattern in positive cells. On morphological analysis by light microscopy the increased immunoreactivity of Ki-M2 and Ki-M3 and the decreased immunoreactivity of Ki-M6 and Ki-M8 were due to a higher proportion of more monocyte-like macrophages. More of these cells had folded or kidney-shaped nuclei and a nuclear/cytoplasmic ratio of 1:1 or 1:2 whereas mature alveolar macrophages possess oval or round nuclei and a nuclear/cytoplasmic ratio >1:3.

Immunostaining with anti-HLA-DR and -DQ revealed a high percentage of alveolar macrophages expressing at least one of the corresponding MHC class II antigens in all subjects with no significant differences found between IPF patients and controls (table 3).

Discussion

Pulmonary fibrosis provides a common endstage of parenchymal destruction caused by a variety of partly unidentified agents [3]. Pathogenetic mechanisms are not yet fully understood. In interstitial lung disease the release of free oxygen radicals is supposed to be a crucial cause leading to progressive fibrosis [3, 5]. It is known that free radicals released by phagocytes can disturb the balance of protease-induced tissue damage [20].

Our study confirmed the results of STRAUSZ *et al.* [7] that alveolar macrophages of IPF patients show increased release of free oxygen radicals. Basic luminol-dependent chemiluminescence and LDCL after stimulation with zymosan were found to be enhanced suggesting augmented tissue injuring capacity of alveolar macrophages in idiopathic pulmonary fibrosis. Additionally we found that free oxygen radical release of blood monocytes is not altered in this disease. Since monocytes are considered as precursor cells of alveolar macrophages [10, 11] this result indicates that functional properties for increased liberation of oxygen metabolites by alveolar macrophages in IPF are acquired in the lungs and not in peripheral circulation.

An increased generation of free oxygen radicals by cells of BAL has also been described in other diseases with involvement of the lungs as shown in sarcoidosis [21] and in rheumatoid arthritis [22]. In contrast to our results in idiopathic pulmonary fibrosis blood monocytes of sarcoidosis patients were reported to display augmented liberation of oxygen metabolites [21] reflecting the fact that sarcoidosis is a disease with systemic involvement.

It is known that peroxidase activity which is required for luminol-dependent chemiluminescence exhibition decreases on a per cell basis during maturation of monocyte/macrophages [23]. Thus, one reason for the stronger LDCL of adherent BAL cells in idiopathic pulmonary fibrosis might be an increased proportion of more immature alveolar macrophages. To verify this, we evaluated the AM stage of differentiation in IPF.

Former studies suggested enzyme cytochemical analysis to be useful in studying the differentiation stage of mononuclear phagocytes [24, 25]. Even more effective tools for this purpose were achieved by developing new monoclonal antibodies. As shown previously [12–14] monoclonal antibodies of the Ki-M-series allow sensitive determination of monocyte/macrophage differentiation stages.

We found alveolar macrophages of controls revealing the phenotype of mature, well-differentiated monocyte/macrophage cells. Immunostaining with Ki-M2 and Ki-M3 showed a weak reactivity whereas Ki-M6 and Ki-M8 displayed a very strong reaction in almost all alveolar macrophages. KREIPE *et al.* [12] and RADZUN *et al.* [13] reported monocytes as precursor cells of alveolar macrophages to display stronger immunostaining with Ki-M2 and Ki-M3, while Ki-M6 and Ki-M8 antigen expression was weaker. Adherent lavage cells from our IPF patients revealed a significantly increased immunostaining with Ki-M2 and Ki-M3 and a significant decrease of Ki-M6 and Ki-M8 positive cells, implying that BAL cells from IPF patients contain more monocyte-like precursor cells of alveolar macrophages.

This was affirmed on morphological analysis. Concerning the fact that LDCL response decreases during differentiation of monocytes into macrophages our results emphasize that increased free oxygen radical release by alveolar macrophages in idiopathic pulmonary fibrosis might at least partially be due to the higher proportion of alveolar macrophages displaying a more monocyte-like immunophenotype in the alveoli of these patients.

MHC class II antigens play an important role in antigen presentation to T lymphocytes. In pulmonary fibrosis a recent report revealed decreased MHC class II antigen expression by a subpopulation of cells in the alveolar capillary endothelial wall, suggesting reduced capacities to present antigens while HLA-DR antigen expression by alveolar macrophages in IPF was reported to be in the normal range [26]. In addition our study revealed normal percentages of HLA-DQ positive AM stressing that in idiopathic pulmonary fibrosis the display of MHC class II antigens is not modified.

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References

1. Hunninghake GW, Garret KC, Richerson HB, Fantone JC, Ward PA, Rennard SI, Bitterman PB, Crystal RG. – Pathogenesis of the granulomatous lung diseases. *Am Rev Respir Dis*, 1984, 130, 476–496.
2. Reynolds HY. – Bronchoalveolar lavage. *Am Rev Respir Dis*, 1987, 135, 250–263.
3. Weissler JC. – Idiopathic pulmonary fibrosis: cellular and molecular pathogenesis. *Am J Med Sci*, 1989, 297, 91–104.
4. Clark RA, Klebanoff SJ. – Studies on the mechanism of antibody-dependent polymorphonuclear leucocyte mediated cytotoxicity. *J Immunol*, 1977, 119, 1412–1418.
5. Nathan C, Cohn ZA. – Role of oxygen-dependent mechanism in antibody induced lysis of tumor cells by activated macrophages. *J Exp Med*, 1980, 152, 198–208.
6. Clement A, Chadelat K, Masliah J, Housset B, Sardet A, Grimfeld A, Tournier G. – A controlled study of oxygen metabolite release by alveolar macrophages from children with interstitial lung disease. *Am Rev Respir Dis*, 1987, 136, 1424–1428.
7. Strausz J, Mueller-Quernheim J, Steppling H, Ferlinz R. – Oxygen radical production by alveolar inflammatory cells in idiopathic pulmonary fibrosis. *Am Rev Respir Dis*, 1990, 141, 124–128.
8. Allen RC, Loose LD. – Phagocytic activation of a luminol dependent chemiluminescence in rabbit alveolar and peritoneal macrophages. *Biochem Biophys Res Commun*, 1976, 69, 245–252.
9. Stevens P, Winston DJ, von Dyke R. – *In vitro* evaluation of opsonic and cellular granulocyte function by luminol-dependent chemiluminescence: utility in patients with severe neutropenia and cellular deficiency states. *Infect Immunol*, 1978, 22, 41–43.
10. Thomas PD, Ramberg RE, Sale GE, Sparks RS, Golde DW. – Direct evidence of a bone marrow-origin of the alveolar macrophage in man. *Science*, 1976, 192, 1016–1018.
11. Radzun HJ, Parwaresch MR, Kreipe H. – Monocytic origin of human alveolar macrophages. *J Histochem Cytochem*, 1983, 21, 318–324.
12. Kreipe H, Radzun HJ, Parwaresch MR. – Phenotypic differentiation patterns of the human monocyte/macrophage system. *Histochem J*, 1986, 18, 441–450.
13. Radzun HJ, Kreipe H, Bödewadt S, Hansmann L, Barth J, Parwaresch MR. – Ki-M8 monoclonal antibody reactive with an intracytoplasmatic antigen of monocyte/macrophage lineage. *Blood*, 1987, 69, 1320–1327.
14. Parwaresch MR, Radzun HJ, Kreipe H, Hansmann ML, Barth J. – Monocyte/macrophage-reactive monoclonal antibody Ki-M6 recognizes an intracytoplasmic antigen. *Am J Pathol*, 1986, 124, 141–151.
15. Boyum A. – Isolation of mononuclear cells and granulocytes from human blood. *Scand J Clin Lab Invest*, 1967, 21, 77–109.
16. Bennet WE, Cohn ZA. – The isolation and selected properties of blood monocytes. *J Exp Med*, 1966, 123, 145–157.
17. Barth J, Welsch U, Schumacher U, Ravens G. – Lectin modified chemiluminescence of human mononuclear cells and morphological correlations to electron microscopy. *In*: Analytical applications of bioluminescence and chemiluminescence. L.J. Kricka, P.E. Stanley, G.H. Thorpe, T.P. Whitehead eds, Academic Press, London, 1984, pp. 381–386.
18. Feller AC, Parwaresch MR, Wacker HH, Radzun HJ, Lennert K. – Combined immunohistochemical staining for surface IgD and T lymphocyte subsets with human monocytes in human tonsils. *Histochem J*, 1983, 15, 557–562.
19. Sachs, L. – *In:* Angewandte Statistik, 6th edn, Springer, Berlin, 1984.
20. Carp H, Janoff A. – *In vitro* suppression of serum elastase inhibitory capacity by reactive oxygen species generated by phagocytosing polymorphonuclear phagocytes. *J Clin Invest*, 1979, 63, 793–797.
21. Barth J, Entzian P, Petermann W. – Increased release of free oxygen radicals by phagocytosing and nonphagocytosing cells from patients with active pulmonary sarcoidosis as revealed by luminol-dependent chemiluminescence. *Klin Wochenschr*, 1988, 66, 292–297.
22. Perez T, Farre JM, Gosset P, Wallaert B, Duquesnoy B, Voisin C, Delcambre B, Tonne AB. – Subclinical alveolar inflammation in rheumatoid arthritis: superoxide anion, neutrophil chemotactic activity and fibronectin generation by alveolar macrophages. *Eur Respir J*, 1989, 2, 7–13.
23. Bigger WD, Sturgess JM. – Peroxidase activity of alveolar macrophages. *Lab Invest*, 1976, 34, 31–42.
24. Barth J, Kreipe H, Kiemle-Kallee J, Radzun HJ, Parwaresch MR, Petermann W. – Diminished activity of tartrate-resistant acid phosphatase in alveolar macrophages from patients with active sarcoidosis. *Thorax*, 1988, 43, 901–904.
25. Radzun HJ, Kreipe H, Parwaresch MR. – Tartrate-resistant acid phosphatase as a differentiation marker for the human mononuclear phagocyte system. *Hematol Oncol*, 1983, 1, 321–327.
26. Komatsu T, Yamamoto M, Shimokata K, Nagura H. – Phenotypic characterization of alveolar capillary endothelial cells, alveolar epithelial cells and alveolar macrophages in patients with pulmonary fibrosis, with special reference to MHC class II antigens. *Virchows Arch*, 1989, 415(1), 79–90.

Les macrophages alvéolaires dans la fibrose pulmonaire idiopathique ont un immuno-phénotype de type plutôt monocytaire et une libération accrue de radicaux libres d'oxygène. J. Kiemle-Kallee, H. Kreipe, H.J. Radzun, M.R. Parwaresch, U. Auerswald, H. Magnussen, J. Barth.

RÉSUMÉ: Un lavage broncho-alvéolaire (BAL) a été exécuté chez 13 patients atteints de fibrose pulmonaire idiopathque, et chez 10 sujets contrôle. La production de radicaux libres d'oxygène (FOR) par les macrophages alvéolaires (AM) et par les monocytes sanguins, a été mesurée par chemoluminescence luminol dépendante (LDCL) avec ou sans stimulation par le zymosan A. Nous avons confirmé les études antérieures montrant que les macrophages alvéolaires, chez le patients atteints de fibrose pulmonaire idiopathique (IPF) ont une libération significativement accrue de FOR dans les conditions basales et après stimulation. Les monocytes sanguins, par contre, n'ont aucune altération de LDCL dans la fibrose pulmonaire idiopathique. Ceci indique que les propriétés fonctionnelles de libération accrue de FOR par les macrophages alvéolaires dans l'IPF sont acquises dans les poumons et non dans la circulation périphérique. Pour élucider les mécanismes possibles conduisant à cette réponse accrue de LDCL, nous avons pratiqué l'immuno-phénotypage des macrophages alvéolaires. Nous avons recouru à une coloration immuno-cytochimique au moyen d'un ensemble de nouveaux anticorps monoclonaux des séries Ki-M, qui discriminent les étapes de différenciation des sous-populations de macrophages et monocytes. Chez les patients IPF, la distribution des AM, Ki-M2, Ki-M3, Ki-M6 et Ki-M8 positifs, a monté une proportion accrue de macrophages alvéolaires, exprimant un immuno-phénotype plutôt monocyte-like. Normalement, les monocytes précurseurs des macrophages alvéolaires ont une LDCL notablement plus forte que les macrophages alvéolaires eux-mêmes. Dès lors, il semble probable que l'augmentation de LDCL des macrophages alvéolaires dans l'IPF est due à une proportion accrue de cellules plus immatures, monocyte-like, dans les alvéoles de ces patients.
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