Virchows Archiv A Pathol Anat (1989) 414:393–397

Virchows Archiv A
Pathological Anatomy and Histopathology

Increased expression of HLA-DR antigen on alveolar macrophages in pulmonary diseases

Wolfgang Popp [1], Otto Braun [2], Hartmut Zwick [1], Helmuth Rauscher [1], and Leopold Ritschka [1]

[1] Pulmonary Department of KH Lainz, Wolkersbergenstrasse 1, A-1130 Vienna, Austria
[2] Department of Pathology, University of Vienna, Spitalgasse 4, A-1090 Vienna, Austria

Summary. The expression of HLA–DR antigen on alveolar macrophages obtained by bronchoalveolar lavage in healthy controls and patients with different diseases was investigated, using cytofluorographic analysis and phykoerythrin conjugated monoclonal mouse anti-human HLA–DR antibody. Alveolar macrophages in patients with extrinsic allergic alveolitis ($n=4$), idiopathic lung fibrosis ($n=4$), sarcoidosis ($n=6$), rheumatoid lung disease ($n=6$) and pulmonary infection ($n=5$) showed increased density of HLA–DR antigen expression compared to healthy control subjects ($n=5$). The increased expression of HLA–DR antigen on alveolar macrophages confirms the importance of these cells for recognition of antigens and immunological responses in different pulmonary diseases.

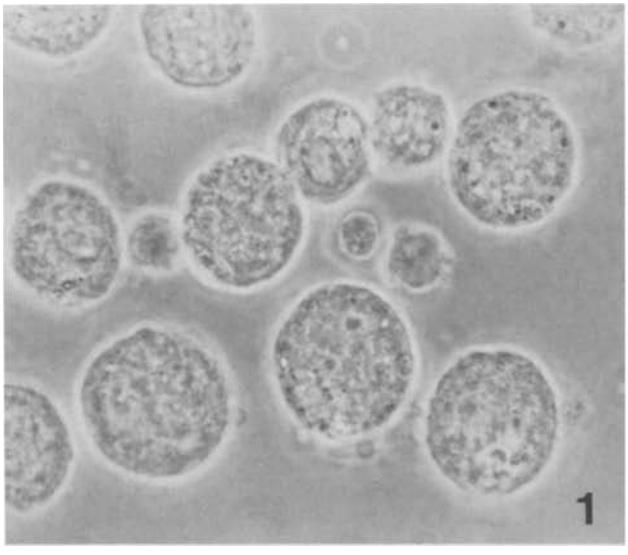
Key words: Alveolar macrophages – HLA–DR antigen – Extrinsic allergic alveolitis – Sarcoidosis – Rheumatoid lung disease

Offprint requests to: W. Popp

Abbreviations: BAL, bronchoalveolar lavage; PBS, phosphate buffered saline

Introduction

The antigen-presenting function of cells is associated with the expression of class II Major Histocompatibility Complex on the cell surface (Janeway et al. 1984; Poulter 1983). Different cells are known to express HLA–DR antigen and thus play an important role in the induction of the immune



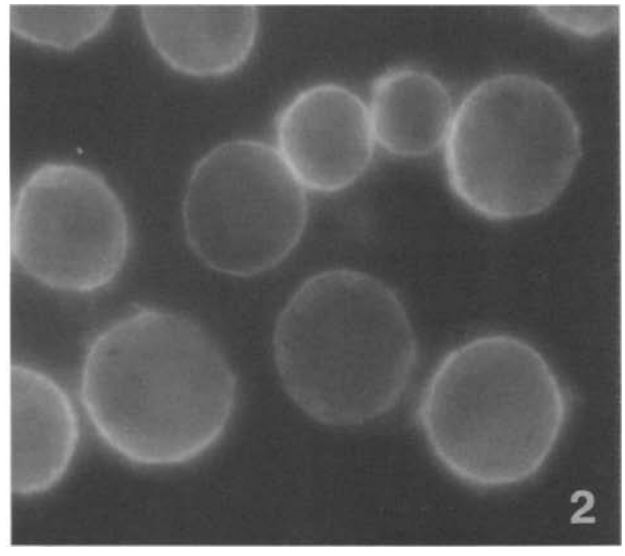


Fig. 1. Phase-contrast photograph of some alveolar macrophages and lymphocytes of patient 13 (× 630)

Fig. 2. Immunofluorescent stained alveolar macrophages: Alveolar macrophages show a wide range of density of HLA–DR antigen expression independent of cell size (× 630)

Table 1. Patients and probands data

Patient No	Age	Sex	Chest X-ray	Lung function (% predicted)			Diversa
				VC	FEV1	TCO/VA	
Extrinsic allergic alveolitis							
1	40	m	nodular interstitial pattern	83	74	61	PA to Micropolyspora
2	50	f		54	54	57	PA to Pullularia
3	59	f		90	84	60	PA to Pullularia
4	69	m	reticulo-nodular	87	78	64	PA to Cephalosporium
Idiopathic lung fibrosis							
5	84	m	reticulo-nodular	94	60	54	–
6	50	m	reticulo-nodular	100	97	80	–
7	49	m	reticular	89	87	76	–
8	65	f	reticular	82	84	78	–
Pulmonary sarcoidosis							
9	32	m	I	110	102	94	ACE 32.1
10	66	f	II	115	84	94	ACE 24.3
11	28	m	I	118	109	88	ACE 28.9
12	30	m	I–II	101	98	80	not done
13	57	f	II	98	69	69	ACE 29.9
14	53	m	I	95	87	89	ACE 35.1
Rheumatoid lung disease							
15	61	f	rheuma nodes	102	72	79	RF pos
16	66	f	normal	114	108	68	RF pos
17	65	f	reticular with pleural thickness	93	90	88	RF pos, ANA pos
18	53	m	reticular	140	123	83	RF pos
19	60	m	reticulo-nodular	71	82	86	RF pos
20	63	m	normal	114	108	67	RF pos
Pulmonary infection							
21	56	m	pneumonic infiltrates	not done			Pneumokokkes
22	30	f	broncho-pneumonic infiltrates	83	57	104	Neisseria
23	34	m	multiple pulmonary abscesses	not done			Staphylokokkes, smoker
24	39	m	broncho-pneumonic infiltrates	not done			Pneumokokkes, smoker
25	62	f	broncho-pneumonic infiltrates	94	85	97	not done
Healthy control subjects							
26	51	m	normal	100	96	96	
27	44	f	normal	109	112	102	
28	62	m	normal	97	99	105	
29	27	f	normal	119	111	113	
30	43	m	normal	120	109	107	

ACE, Serum angiotension converting enzyme (normal range 7–26 U/ml); ANA, Antinuclear antibodies; Ches X-ray of sarcoidosis: I = bilateral hilar lymphadenopathy; II = I + infiltrates; III = lung fibrosis; FEV1, Forced expiratory volume in 1 s; PA, Precipitating antibodies; RF, Rheumatoid factors; TCO/VA, Transfer factor for carbon monoxide corrected for alveolar volume; VC, Vital capacity

response (Reinherz et al. 1981; Schuler et al. 1983). The density of HLA–DR antigen expression on antigen-presenting cells is correlated with the function of primary immune response (Janeway et al. 1984; Saltini et al. 1985).

Bronchoalveolar lavage (BAL) is an established method and an excellent tool for investigation of immunological diseases of the lung (Costabel et al. 1985; Reynolds 1987). In normal subjects, the majority of alveolar macrophages expresses HLA–DR antigen. Relative and absolute numbers of HLA–DR positive alveolar macrophages obtained by BAL differ between various pulmonary diseases (Venet et al. 1985; Razma et al. 1984; Crystal et al. 1986). Increased density of HLA–DR antigen expression on alveolar macrophages was recently shown in sarcoidosis (Campbell et al. 1986).

This study aimed to determine the proportion of HLA–DR antigen bearing alveolar macrophages and the density of this antigen in healthy control subjects and in patients suffering from pulmonary diseases. Using cytofluorometric analysis

Table 2. Results from BAL

Patient	Diagnosis	BAL recovery (%)	Cells/ml ($\times 10^3$)	Lymphocytes	Neutrophils	Eosinophils	Macrophages
1	EAA	80	410	76	3	1	20
2	EAA	40	588	51	3	15	31
3	EAA	75	387	49	34	2	15
4	EAA	60	210	74	0	1	25
5	ILP	50	468	2	0	0	98
6	ILP	75	163	6	5	0	89
7	ILP	70	436	10	11	0	79
8	ILP	65	272	32	9	1	58
9	PS	76	360	49	1	0	50
10	PS	75	133	35	1	2	62
11	PS	66	272	42	1	0	57
12	PS	90	356	36	1	1	62
13	PS	42	119	44	2	1	53
14	PS	50	204	71	0	0	29
15	RLD	85	553	1	1	1	97
16	RLD	60	207	31	2	0	67
17	RLD	65	219	41	8	4	47
18	RLD	89	88	16	3	0	81
19	RLD	60	185	25	11	7	57
20	RLD	53	215	21	2	0	77
21	PI	45	251	44	4	0	52
22	PI	75	250	21	3	0	76
23	PI	60	733	30	21	1	48
24	PI	80	500	17	60	1	22
25	PI	35	252	22	47	5	26
26	H	56	236	1	2	1	96
27	H	55	136	1	1	0	98
28	H	45	89	10	2	0	88
29	H	75	167	6	4	0	90
30	H	65	206	2	5	1	92

EAA, Extrinsic allergic alveolitis; ILP, Idiopathic lung fibrosis; PS, Pulmonary sarcoidosis; RLD, Rheumatoid lung disease; PI, Pulmonary infection; H, Healthy control subjects

we were able to detect small differences in HLA–DR antigen expression under different conditions.

Materials and methods

Four patients with allergic alveolitis, four patients with idiopathic lung fibrosis, six patients with pulmonary sarcoidosis, six patients with rheumatoid lung disease, five patients with pulmonary infection and five healthy non-smoking control subjects underwent BAL and afterwards transbronchial lung biopsy for confirmation of clinically, radiologically and serologically suspected diagnosis (see Table 1). BAL was performed during topical anaesthesia with 2% xylocaine via a fiberbronchoscope (Olympus B3) which was wedged in the medial segment of middle lobe. A total of 100 ml 37° C steril isotone saline was installed in 20 ml aliquots and recovered by gentle aspiration. BAL fluid was collected into siliconized glass tubes and processed immediately at 4° C.

Viability of more than 90% of cells from BAL was shown with Trypan Blue staining. Cell count was done in a haemocytometer on unconcentrated BAL fluid and expressed as cells per milliliter. Differential cell count was performed on 400 cells from a cytocentrifuged cell preparation stained with May-Grünwald-Giesma. One milliliter of BAL fluid was incubated with 25 µl PBS (pH 7.3) another with 25 µl monoclonal mouse anti-human OKDR-phykoerythrin conjugate (Ortho Diagnostics) at 4° C for 30 min. Afterward non-specific bindings were removed by washing in PBS (pH 7.3).

For the detection of HLA–DR antigen bearing alveolar macrophages and quantification of density of HLA–DR antigen expression cell and fluorescence analysis was done with an Ortho-Spectrum III cytofluorometer. Alveolar macrophages were gated in forward and right scattered ion argon laser-light (488 nm) and autofluorescence was measured by means of red fluorescence high pass filter (630 nm) (Popp et al. 1988). Cell size and immunofluorescence of stained alveolar macrophages was then measured under the same conditions and expressed in relative immunofluorescence units. A minimum of 1000 cells of each probe was evaluated. HLA–DR antigen bearing alveolar macrophages were expressed as the percentage of total alveolar macrophages. Calculation of the mean density of immunofluorescence and its standard deviation was done. Immunofluorescent staining on cytocentrifuge preparations was controlled by means of fluorescence microscopy (Figs. 1 and 2).

HLA–DR antigen positive and negative alveolar macrophages were compared using Brandt Snedecor's chi-square test. For other statistical analyses the Student-*t*-test and the Kolmogoroff Smirnoff test (Sachs 1984) was used. A p value of <0.01 was regarded as statistical significant. All methods were investigated for precision and accepted as reproducible if the coefficient of variation was <0.05.

Table 3. Immunofluorescence results of alveolar macrophages

Patient No	Cell size (diameter in μm)	HLA–DR positive alveolar macrophages (%)	Immunofluorescence "units"	Immunofluorescence "units" per μm^2 surface
Extrinsic allergic alveolitis				
1	23.5±7.7	98	168±41**	0.0969±0.0471**
2	26.4±7.7	98	139±33**	0.0635±0.0330**
3	21.5±8.4	97	124±31**	0.0854±0.0302**
4	25.6±7.1	96	87±26**	0.0423±0.0145**
Idiopathic lung fibrosis				
5	26.3±5.6	97	104±33**	0.0479±0.0053**
6	24.5±5.0	95	105±44**	0.0557±0.0046**
7	27.6±5.2	98	112±35**	0.0468±0.0033**
8	27.3±5.5	96	124±44**	0.0530±0.0034**
Pulmonary sarcoidosis				
9	25.5±4.4	96	76±25	0.0372±0.0012
10	24.0±5.7	96	85±36**	0.0470±0.0033**
11	26.5±5.0	95	115±35**	0.0522±0.0041**
12	22.1±4.3	97	77±29	0.0502±0.0020**
13	26.9±6.5	99	131±37**	0.0577±0.0142**
14	28.0±5.4	98	99±32**	0.0402±0.0028**
Rheumatoid lung disease				
15	23.5±4.4	96	97±35**	0.0559±0.0023**
16	24.2±4.8	98	111±42**	0.0604±0.0029**
17	24.8±5.9	97	102±29**	0.0528±0.0116**
18	25.7±6.1	95	89±36**	0.0429±0.0035**
19	22.9±7.6	97	131±37**	0.0796±0.0360**
20	27.2±5.2	92	96±29**	0.0413±0.0034**
Pulmonary infection				
21	23.5±7.8	99	167±41**	0.0963±0.0487**
22	26.9±6.3	96	129±31**	0.0568±0.0160**
23	25.7±6.0	95	90±27**	0.0434±0.0088**
24	25.6±6.9	94	86±35**	0.0418±0.0052**
25	25.2±5.9	95	91±37**	0.0456±0.0035**
Healthy control subjects				
26	26.4±5.0	95	82±28	0.0375±0.0020
27	24.6±5.3	97	73±26	0.0384±0.0031
28	24.5±4.2	94	71±25	0.0377±0.0018
29	25.6±5.2	91	73±24	0.0355±0.0029
30	25.7±5.0	94	80±24	0.0386±0.0035

Statistical analysis of immunofluorescence results were done to the healthy control subjects. * indicates a $p<0.01$; ** indicates a $p<0.001$

Results

Patients' data are given in Table 1, and differential cell counts obtained from BAL fluid in Table 2. Immunofluorescent staining with phykoerythrin conjugated monoclonal antibody and cytofluorographic analysis by a laser flow cytometer gave excellent results. Autofluorescence was minimized using red fluorescence high pass filter and by this the sensitivity of the immunofluorescent staining became higher. Reliability of this method was guaranteed by high cell count of each sample.

Immunofluorescence results of HLA–DR antigen expression on alveolar macrophages are shown in Table 3. HLA–DR antigen bearing alveolar macrophages were slightly increased in each group of patients. Immunofluorescence per cell was increased in all but two patients regardless of the underlying disease ($p<0.01$). Only two patients with sarcoidosis (no 9 and 12) had mean immunofluorescence per cell not different from healthy control subjects. Regarding the mean density of HLA–DR antigen expression (immunofluorescence per μm^2 cell surface) all groups of diseases had elevated values ($p<0.01$). Although large alveolar macrophages had higher degree of HLA–

DR antigen expression per cell, increased density of HLA–DR antigen was not seen on these cells.

Discussion

Alveolar macrophages and other macrophages and dentritic cells are responsible for antigen presentation (Stingl et al. 1978; Poulter 1983; Du Bois 1985; Ettensohn et al. 1986). HLA–DR antigen expression, a class II Major Histocompatibility Complex, is known to be important in this pathway of immune response (Benacerraf 1981; Janeway et al. 1984; Unanue et al. 1984; Venet et al. 1985). Augmented antigen presentation in sarcoidosis was found to be due to an increased percentage of HLA–DR or HLA–DS antigen expression on alveolar macrophages (Venet et al. 1985). Furthermore, the density of HLA–DR antigen expression on alveolar macrophages is increased in pulmonary sarcoidosis (Campbell et al. 1986). Activation of alveolar macrophages is also seen in the majority of interstitial lung diseases (Cohen and Cline 1971; Du Bois 1985). These activated alveolar macrophages are very likely to express more HLA–DR antigens.

This investigation demonstrates an increased HLA–DR antigen expression on alveolar macrophages in different pulmonary diseases. There is a high number of HLA–DR bearing alveolar macrophages and a wide range of HLA–DR antigen expression on individual alveolar macrophages. Although the range of HLA–DR antigen expression on individual alveolar macrophage is wide, significant differences in HLA–DR expression were found between healthy and diseased subjects. Different mechanisms and intensities of stimulation by immunological or infectious diseases as well as differences in the individual reagibility of alveolar macrophages may explain these findings.

As soon as BAL became a routine method for investigation of interstitial lung diseases, more information concerning biological mechanisms of alveolar macrophages became available concerning their activation and its relationship with lymphocyte proliferation. Monitoring of alveolar macrophage activation may be a sensitive tool in the management and treatment of interstitial lung diseases, helping to prevent ongoing lung injury and subsequent fibrosis.

Acknowledgement. The authors would like to thank MTA E. Handler, B. Schreiber and E. Holzgruber for their technical assistance, and to Mrs. H. Weber for typing the manuscript.

References

Benacerraf B (1981) Role of MCH gene products in immune regulation. Science 212:1229–1238

Campbell DA, Du Bois RM, Butcher RG, Poulter LW (1986) The density of HLA–DR antigen expression on alveolar macrophages is increased in pulmonary sarcoidosis. Clin exp Immunol 65:165–171

Cohen AB, Cline MJ (1971) The human alveolar macrophage: Isolation, cultivation in vitro, and studies of morphologic and functional characteristics. J Clin Invest 50:1390–1398

Costabel U, Bross KJ, Matthys H (1985) Bronchoalveoläre Lavage: Klinische Bedeutung zytologischer und immunzytologischer Befunde. Prax Klin Pneumol 39:343–355

Crystal RG, Reynolds HY, Kalica AR (1986) Bronchoalveolar lavage – The report of an international conference. Chest 90:122–131

Du Bois RM (1985) The alveolar macrophage. Thorax 40:321–327

Ettensohn DB, Lalor PA, Roberts NJ jr (1986) Human alveolar macrophage regulation of lymphocyte proliferation. Am Rev Respir Dis 133:1091–1996

Janeway CA, Bottomly K, Babich J, Conrad P, Conzen S, Jones B, Kaye J, Katz M, McVay L, Murphy DB, Tite J (1984) Quantitative variation in Ia expression plays central role in immunoregulation. Immunol Today 5:99–105

Popp W, Braun O, Zwick H, Ritschka L, Rauscher H (1988) Immunzytologische und immunhistochemische Nachweismethoden bei der exogen allergischen Alveolitis. Prax Klin Pneumol 42:549–550

Poulter LW (1983) Antigen presenting cells: they identification and involvement in immunopathology. Clin exp Immunol 53:513–520

Razma AG, Lynch JP, Wilson BS, Ward PA, Kunkel SL (1984) Expression of Ia-like (DR) antigens on human alveolar macrophages isolated by bronchoalveolar lavage. Am Rev Resp Dis 129:419–424

Reynolds HY (1987) Bronchoalveolar lavage. Am Rev Respir Dis 135:250–263

Sachs L (1984) Angewandte Statistik. 6th ed. Springer, Berlin

Saltini C, Spurzem JR, Crystal RG (1985) Is the exaggerated T-cell alveolitis of sarcodosis associated with an inability of T suppressor cells to proliferate? Am Rev Resp Dis 131:18A

Schuler G, Stingl G, Aberer W, Stingl-Gazze LA, Hönigsmann H, Wolff K (1983) Histiozytosis X cells in eosinophilic granuloma express Ia and T6 antigens. J Invest Dermatol 80:405–409

Stingl G, Katz SI, Clement L, Green I, Shevach EM (1978) Immunologic functions of Ia-bearing epidermal Langerhans cells. J Immunol 121:2005–2013

Unanue ER, Beller, DI, Lu CY, Allen PM (1984) Antigen presentation: comments on its regulation and mechanism. J Immunol 132:1–5

Venet A, Hance AJ, Saltini C, Robinson BW, Crystal RG (1985) Enhanced alveolar-macrophage mediated antigen-induced T lymphocyte proliferation in sarcoidosis. J Clin Invest 75:293–301

Received October 17, 1988 / December 27, 1988