STUDY ON PROTEIN FACTOR IN ALVEOLAR MACROPHAGE OF EXPERIMENTAL SILICOSIS

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The immunological theory of silicosis suggested by Vigliani et al opened a new way for studies on the pathogenesis of silicosis.[2] Previous researches on biochemical, pathological, and immunological changes in silicosis showed that immunological factors were involved in the producing and developing process of silicosis,[3] and that alveolar macrophage (AM) phagocytosis and destruction were important in the pathogenesis of silicosis. In this paper, the research results of a protein factor (PF) in AM of experimental silicosis were reported. It may be of practical significance for early diagnosis and further understanding of the pathogenesis of silicosis.

Conventional methods were used for animal models coping, dust particle preparation, dust instillation, observational indices and pathological, biochemical, immunological examinations.[4,5,6]

1. *Detection of serum protein factor (SPF) and the relationship between this SPF and the lesion in silicosis.* The anti-silicotic-rat lung rabbit serum (ASLS) and double agar diffusion method were used in the tests. Totally 243 serum samples from 53 silicotic rats and 28 normal rats were examined. The results showed that a SPF existed in the serum of silicotic rats, and between this serum and anti-serum a precipitation line was observed. The positive rate of SPF in silicotic rats was up to 82.8%, while the results were all negative for normal rats.

Different kinds of dust particles were injected into rat lungs. By checking the serum reaction in animals, it could be found that the positive rate of SPF in silicitic rats was closely related to the pathogenetic effect of dust. Higher positive rate was observed for more pathogenetic dust, and vice versa. (see Table I)

SPF positive rate increased with the periods after intratracheal injection of dust. Seven days after injection, SPF was negative; on the fifteenth day the positive rate increased up to 7.7%, and it was as high as 61.5%, 72.7%, 83.0%, and 100%, respectively after 30, 60, 180 and 210 days.

In order to study the dependence of SPF positive rate of silicotic rats on the silicosis lesion, 109 rats were divided into five groups according to wet weight of the lungs. Serum reactions, pathological changes and the amount of lung collagen were compared, and the results showed that SPF positive rate was in consistence with the extent of lesion. (see Table II)

It was also found that SPF positive rate decreased in some degree with the alleviation in silicotic lesion after therapy with PVNO and other drugs in our experiments. (see Table III)

For understanding the SPF level of rats intratracheally injected with quartz dust, 98 serum samples from 22 dusted

Table I

Relationship between SPF Positive Rate and Dust Property

dust	animal numbers	positive numbers	positive rate(%)	mean wet weight of rat lungs (g)
SiO_2	47	34	82.98	6.3
CaF	4	1	25.00	2.8
SiO_2+Na_2F	10	2	20.00	4.7
SiO_2+$Al(OH)_3$	11	4	36.36	4.9

Table II
Relationship between SPF Positive Rate and Silicotic Lesion

Wet weight of rat lungs(g)	animal numbers	positive rate (%)	pathological grade(IV-V%)	content of collegen in whole lung (mg)
3	4	0·0	0.0	63.6
3–4	35	14.3	43.3	130.2
5–6	52	67.3	59.9	184.2
7–8	15	93.3	63.6	247.9
9	5	100.0	70.0	306.3

Table III
Relationship between SPF Positive Rate and Curative Effect of Drugs

groups	animal numbers	positive rate(%) before treatment	positive rate(%) after treatment	wet weight of rat lung (g)	pathological grade
PVNO prevented	5	-	0.0	2.9	0.0
PVNO treated	20	95.0	0.0	4.3	38.7
anti-silica 14	13	76.9	50.0	5.3	70.7
SiO_2(control)	15	92.8	77.7	6.3	73.0

rats were examined from 45 to 180 days after chest, peritoneal, subcutaneous, intraveneous injection, and SPF were all found negative, while SPF positive rate after intratracheal injection was as high as 72.7% to 83.9%.

2. *Relationship between SPF and AM—Study on the source of SPF.* It is of practical importance to study on the source of SPF for finding a simple, reliable method to diagnose the early silicosis and to define a curative effect index. Hence, we prepared an anti-silicotic-rat AM rabbit serum (ASMS) and investigated its reaction with SPF.

   Through light microscope observations of silicosis pathology and tests about PVNO treatment, it was found that the SPF positive rate was closely related to the destruction of dusted AM.

   Cytotoxicity test showed that ASLS, ASMS had significant cytotoxic effects on the AM of dusted rats when complements existed in vitro. About 75 to 99 percent of AM was coloured by trypan blue.

   Agglutination reaction showed that ASLS, ASMS had higher agglutination effect on AM of dusted rats and the agglutination title were 1 640 and 1 1280, respectively.

   During agar diffusion precipitation processes, when ASLS and ASMS reacted with the same reactant (serum or lung homogeneity of dusted rats) it was found that two precipitation lines presented pattern of fusion. Precipitation lines formed by the same anti-serum and different reactants fused with each other.

3. *Dynamics of AM numbers in the developing of experimental silicosis.* Using Myrvik's method, AM of rats was collected via bronchus alveolar lavage.[1] By counting the cell numbers, AM numbers of dusted and normal rats were compared and the results showed that the former was much more than the latter. Mean AM numbers of the dusted and normal rats were 169.2 and 38.1 $\times 10^6$ respectively.

   The number of AM at different time after exposure to quartz dust was observed. The results indicated that AM numbers increased with the time. Mean AM numbers were counted to be 57.8, 163.9, 102.9, 175.1, and 347.4 ×

10^6 in 15, 30, 60, 90 and 130 days after exposure of dust. Whereas at each time AM numbers markedly differed from that in normal rats (38.1×10^6).

Wet weight of the lung, lesion extent and lesion hardness were usually used as indices for the degree of silicotic pathological changes. If the silicotic rats were grouped according to wet weight, it could be observed that the increase in AM numbers was in accordance with the increase in wet weight. For instance, AM numbers was 58.6×10^6 for wet weight less than 4 g, the numbers increased to 171.2×10^6 for wet weight of 4–5 g, and it was 239.9×10^6 when the latter was more than 5 g.

If the rats were grouped by AM numbers, it would be found that there was correspondence between AM numbers and extent or hardness of lesion. (Table IV)

DISCUSSION

The serum of experimental silicotic rats may react with ASLS, thus forming a clear precipitation line on the agar base. This indicated that a certain SPF existed in both silicotic rat serum and silicotic rat lung. In the serum of normal rats, due to the lack of the SPF or the small quantity, if there was such PF, it was not detected. SPF positive rate was proportional to the pathogenetic effect of dust, time prolongation after exposure of dust and the extent of silicotic lesion, so this rate might reflect the seriousness of experimental silicotic lesion. SPF positive rate would decrease due to the utilization of effective drugs. In general, the method is characterized by easy operation and can be applied to dynamic observations to rats, and is appropriate to be used as an index of curative effect for experimental silicosis.

Researches on the morphological changes of AM on the tissue section and on the effect of PVNO treatment demonstrated that there was correspondence between SPF positive rate and AM lesion. As the time after exposure to dust increased, AM was gradually destroyed, meantime the lesion progress with the presence of quartz dust. During the process PF was released and accumulated, its content into blood from lung increased. For this reason SPF positive rate grew.

Control experiments using ASLS and ASMS showed that both anti-serums were cytotoxic to the AM of silicotic rats and caused the AM to agglutinate in vitro. During the agar diffusion tests, the precipitation lines formed between ASLS or ASMS and silicotic rat serum, AM or lung homogenecity fused with each other. The silicotic rat SPF detected by both anti-serums were located in α-globulin position and the precipitation curves tended to fuse. Based on the above mentioned results, we could draw the conclusion that silicotic rat SPF and the destruction of dusted AM were closely related.

The consistent increase of AM reflected the continuous death of macrophages. It is assumed that the following methods can be used for prevention and treatment of silicosis: inhibiting the source of local AM for decrease of phagocytosis; restraining the phagocytotic ability of local AM for reduction of the number of destroyed macrophages: stabilizing the lysosome membrane for protecting AM from quartz dust.

CONCLUSIONS

1. In the serum of silicotic rats a PF was found, the positive rate which is proportional to lesion seriousness, lung fibrogenic extent and the extent of AM destruction. It is also inhibited by some effective drugs.
2. The PF in the serum of silicotic rats was located in α-globulin position and originated from dusted AM.
3. The number of AM in dusted rats was much more than that in normal rats. It increased with time after exposure of dust and was directly proportional to the silicotic lesion.
4. Experimental results showed that AM destruction played an important role in the developing of experimental silicosis. It is believed that silicotic lesion may be detected and evaluated by ASMS, thus a new way to study the early diagnosis, presentation and treatment of experimental silicosis will be provided.

Table IV

Relationship between AM Numbers and Silicotic Lesion

AM numbers ($\times 10^6$cells)	animal numbers	numbers lung of leaves	lesion extent(%) 0	+	++	+++	lesion hardness(%) 0	+	++	+++
100	7	70	30.1	57.1	11.4	1.4	330.0	41.4	24.3	4.3
100–200	6	60	10.0	38.3	36.7	15.0	10.0	41.6	21.7	26.7
200	7	70	2.9	45.7	22.9	28.5	2.8	47.1	45.6	4.5

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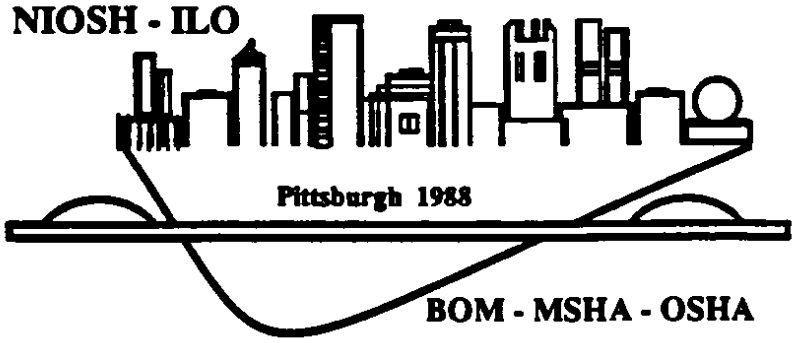
Proceedings of the VIIth International Pneumoconioses Conference *Part*
Transactions de la VIIe Conférence Internationale sur les Pneumoconioses *Tome*
Transaciones de la VIIa Conferencia Internacional sobre las Neumoconiosis *Parte*

II



Pittsburgh, Pennsylvania, USA—August 23–26, 1988
Pittsburgh, Pennsylvanie, Etats-Unis—23–26 aout 1988
Pittsburgh, Pennsylvania EE. UU—23–26 de agosto de 1988

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November 1990

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DHHS (NIOSH) Publication No. 90-108 Part II