

Immunohistochemical Analyses of Masson Bodies in Organizing Pneumonia Pattern in Interstitial Pneumonias

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Conflict-of-interest statement: The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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Received: March 25, 2021

Revised: April 20, 2021

Accepted: April 22, 2021

Published online: May 6, 2021

ABSTRACT

To clarify the intimate relationship between the formation of Masson bodies (MBs) and alveolar mouths (AMs), we carried out detailed immunohistochemical analyses of MBs from 17 cases with various kinds of pneumonia. The cases comprised idiopathic or secondary organizing pneumonia (OP). Immunohistochemical analyses revealed the heterogeneous components of each MB in each individual case of pneumonia. Initially, the thin epithelial cells of AMs were damaged

by endogenous or exogenous stimuli, and tissue exudate, fibrin, cell debris, macrophages, and aspirated materials from the airway were gradually organized revealing continuity with AMs. The MBs comprised surface alveolar epithelial cells, which were extended by the mitoses of the alveolar epithelial cells including those of AMs, and maturational processes, such as Krebs von den Lungen-6 (KL-6) and surfactant protein A (SP-A) positivity, occurred on their surfaces. Blood vessels, mesenchymal interstitial cells, fibroblastic cells, and some inflammatory cells extended into the MBs through the AMs. The MBs contained collagen fibers produced by fibroblasts, but not elastic fibers. Myofibroblasts were concentrically arranged in the MBs, which were connected directly with the AMs, and they transformed into collagen fiber-producing fibroblasts. Therefore, AMs may play an important role in the formation of MBs in OP patterns of (interstitial) pneumonia.

Key words: Organizing pneumonia; Masson body; Immunohistochemistry; Alveolar mouths

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Ohtsuki Y, Fujita J, Horie Y, Iguchi M, Kurabayashi M, Furihata M. Immunohistochemical Analyses of Masson Bodies in Organizing Pneumonia Pattern in Interstitial Pneumonias. *Journal of Respiratory Research* 2021; 7(1): 158-162 Available from: URL: <http://www.ghrnet.org/index.php/jrr/article/view/3120>

INTRODUCTION

The organizing pneumonia (OP) pattern is commonly found in various kinds of interstitial pneumonia (IP), such as non-specific IP (NSIP), cryptogenic organizing pneumonia (COP), collagen vascular disease-related IP, drug-induced IP, and aspiration-associated pneumonia. The Masson bodies (MBs) in these IPs are connected with alveolar mouths (AMs), which constitute a very important locus for the formation of MBs and hyaline membranes (HMs), as reported^[1]. The initial damage of the thin epithelium of AMs is very important because it is the initial stage in the formation of HMs and MBs. We have reported the importance of this damage to HM formation^[1]. With regard to MBs, the importance of both epithelial damage in the peripheral airway^[2] and of cell-matrix patterns^[3] has been

reported. Among the components of MBs, blood vessels proliferate with the accompanying production of vascular endothelial growth factor (VEGF)^[4] and/or basic fibroblast growth factor (bFGF)^[5] by myofibroblasts. However, to date no detailed examination has been reported for each component of MBs, especially with regard to the extending pattern of the covering epithelium and blood vessels with maturational processes that extend into the MBs.

In this report, we focused on MBs of which the covering epithelial cells were directly connected with the alveolar epithelial cells at the early stage of their formation, and of which each aspect, especially epithelialization, proliferating cells within MBs and blood supply, was investigated in detail in relation to AMs.

MATERIALS AND METHODS

A total of 17 cases were examined. There were eleven males and six females (aged 36-96 years). Pulmonary tissue was obtained by video-assisted thoracoscopy (VATS) from eight cases and by autopsy from eight cases. These 17 cases comprised five cases of NSIP/OP, one case of acute fibrinous organizing pneumonia (AFOP), three cases of collagen vascular disease-related interstitial pneumonia (CVD-IP), three cases of secondary OP followed by aspiration pneumonia, two cases of drug-induced interstitial pneumonia, two cases of cancer-associated OP and one case of bronchiectasis with OP as listed in Table 1.

All patients for VATS resection provided written informed consent, and their identities have been strictly protected. As for the autopsied cases, it was stated in the certificate of autopsy permission, that the materials were able to use for the medical education and the medical research in case. Therefore, to use the materials of both surgical and autopsied materials in this study were no problem.

In each case, Masson body-containing representative sections were cut (4- μ m thick), dewaxed, and stained with hematoxylin and eosin (H&E). For special stains, Alcian blue stain (pH2.5) was used for the matrix, phosphotungstic acid-hematoxylin (PTAH) was used for fibrin, and elastica van Gieson (EVG) stain was used for the elastic fibers.

An immunohistochemical study was performed using the antibodies listed in Table 2, as reported previously^[1,6]. Target cells or tissue of each antibody in MBs were shown in Table 3. Each section to be investigated was stained using Ventana BenchMark GX (Roche Diagnostic Co., Ltd., Tokyo, Japan) following the manufacturer's protocol. Only few cases were stained using the LSAB2 kit/horseradish peroxidase (HRP) (DakoCytomation, Kyoto, Japan) method, following the instructions provided in the kit manual.

RESULTS

Ordinary form of MBs connected with AMs (arrowheads) were shown in Figure 1A-E. The epithelial cells covered the exudates from damaged AMs to form MBs (Figure 1A), some containing inflammatory cells, and comprising an abundant alcian blue-positive myxoid matrix (Figure 1B), connecting directly with the AMs (Figure 1C, arrow). When the covering epithelial cells of MBs were stained with CK19, positive epithelial cells were found surrounding the MBs (Figure 1D), but others were incomplete or non-covering (Figure 1E). The similar patterns were obtained with AE1/AE3 (pan-cytokeratin) antibody. At the early stage of MBs, no covering epithelial cells were detected. MBs grew to fill up the air space without containing elastic fibers, implying that the fundamental pulmonary structures were not destroyed. The newly-formed epithelial layers on MBs were a continuation of TTF-1-

Table 1 Clinicopathological findings of the 17 cases examined.

	Age	Sex	Pathological diagnosis	Other findings	VATS / Autopsy
1	78	F	NSIP/OP ^a		VATS
2	70	F	NSIP/OP	ANCA(+)	VATS
3	66	F	NSIP/OP	ANCA(+)	VATS
4	72	F	NSIP/OP		VATS
5	55	M	NSIP/OP	Microscopic HC ^f	Autopsy
6	79	M	AFOP ^b	Markedly organized	Autopsy
7	57	F	RA ^c -IP(NSIP/OP)		VATS
8	76	M	RA-IP(UIP/NSIP)	AE ^g (DAD ^h +OP)	Autopsy
9	78	F	PM ^d -OP		VATS
10	36	M	Secondary massive OP	Bronchiectasis	VATS
11	87	M	Secondary massive OP	Aspiration pn	Autopsy
12	68	M	Secondary massive OP	Aspiration pn	Autopsy
13	96	M	Secondary massive OP	Aspiration pn	Autopsy
14	54	M	Drug-induced IP		VATS
15	54	M	Drug-induced IP		Autopsy
16	65	M	SmCC ^e + UIP type IP	AE (DAD+OP)	Autopsy
17	73	M	Adenocarcinoma+ Focal OP		Lobectomy

^a Non-specific interstitial pneumonia/organizing pneumonia; ^b Acute fibrinous and organizing pneumonia; ^c Rheumatoid arthritis; ^d Polymyositis; ^e Small cell carcinoma; ^f Honeycombing; ^g Acute exacerbation; ^h Diffuse alveolar damage; ⁱ Video-assisted thoracoscopy.

Table 2 Antibodies used in this study.

Antibodies(clone)	Dilution	Pretreatment	Manufacturer
KL-6	1:2,860	NP ^a	Provided by Eisai Co., Tokyo, Japan
EMA(E29)	1:50	MW ^b	Dako, Kyoto, Japan
AE1/AE3(pan-cytokeratins)	0.319	P ^c	Boehringer Mannheim, Germany
CK19(RCK108)	1:30	MW	ScyTek Lab, USA
Alpha-SMA(polyclonal)	1:50	NP	Dako, Kyoto, Japan
SP-A(PE10)	0.111	AC ^d	Dako, Kyoto, Japan
SP-D(10H11)	1:1,600	MW	Provided by Yamasa, Choushi, Japan
CD34(QBEnd 10)	1:10	P	Becton Dickinson Biosciences, USA
CD68(KP-1)	0.458	MW	Nichirei, Biosciences, Inc., Tokyo, Japan
CD20(L26)	1:50	MW	Dako, Kyoto, Japan
CD79a(JCB117)	0.111	AC	Dako, Kyoto, Japan
Factor VIII related antigen (polyclonal)	0.319	NP	Dako, Kyoto, Japan
Collagen type IV(CIV22)	1:50	P	Dako, Kyoto, Japan
MIB-1(Ki-67)	1:50	AC	Dako, Kyoto, Japan

^a NP: non-pretreatment; ^b MW: microwave; ^c P: pronase-pretreated; ^d AC: autoclave.

positive alveolar cells, as revealed direct connection with AMs (Figure 2A, arrows). MBs were often formed through the pores of Kohn, with no epithelialization. When massive MBs were formed in a short time, they were not covered by epithelial cells (Figure 1C), and after epithelial cells on the surface of the MBs extended from the AMs. KL-6 appeared linearly on their surface very early, and connected with the thin alveolar septa. Blood vessels also passed through the AMs and extended into the MBs, as shown by CD34- or collagen type IV- (Figure 2B) positive blood vessels in the MBs. Each antibody reacted with the endothelial cells and the basal lamina respectively. Factor VIII-related antigen also stained vascular endothelial cells within MBs similar to CD34.

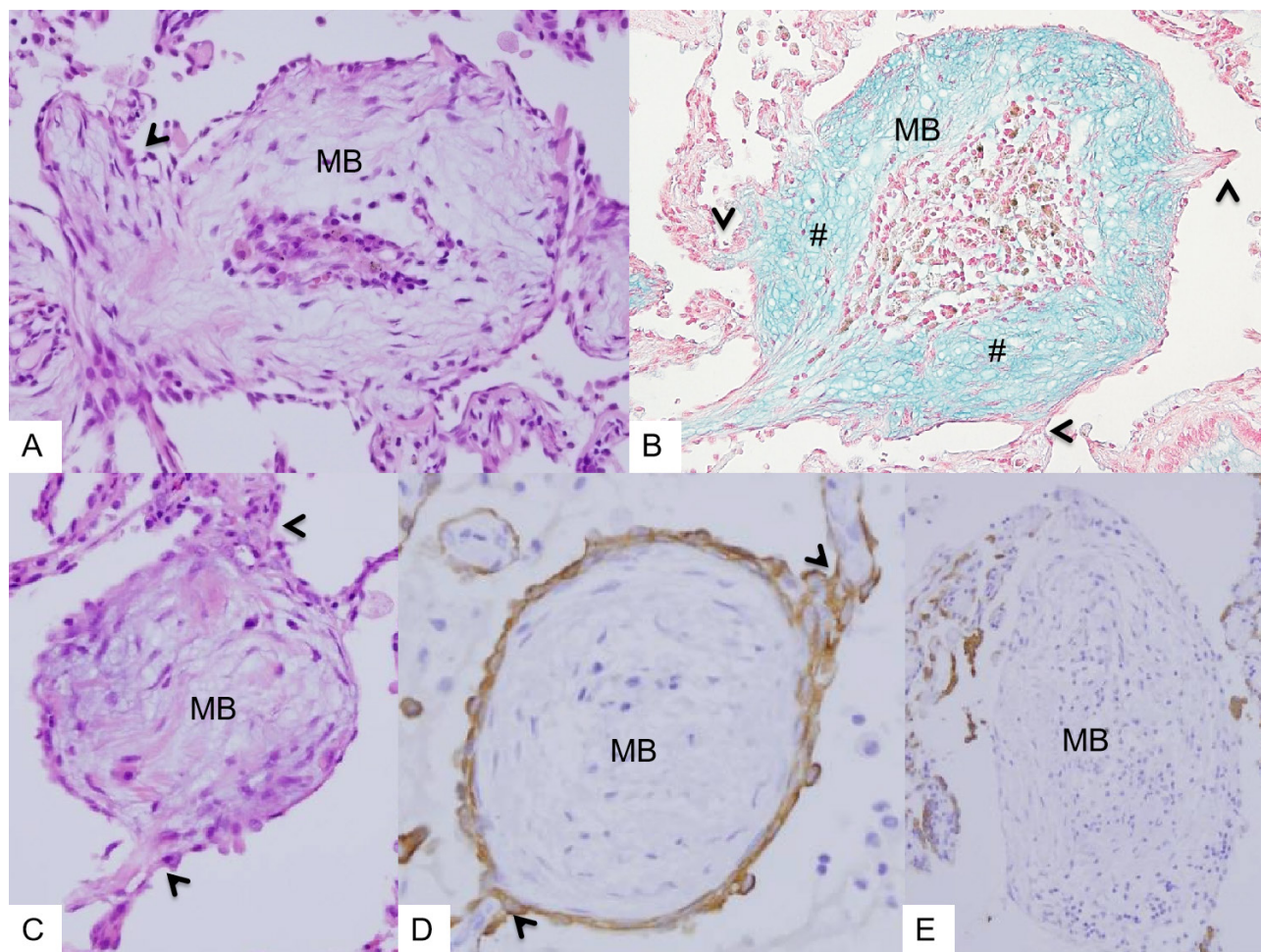


Figure 1 A: Masson body (MB) containing myxoid matrix. B: Alcian blue(AB) -positive matrix in MB (#). C: MB connecting to alveolar mouths (AMs). D: CK19-positive epithelium. E: MB with no epithelium. A&C: HE stain, B: AB stain, A-E: X200, AMs are indicated by arrowheads.

MBs contained alpha-smooth muscle actin (α -SMA)-positive cells, namely myofibroblasts, but α -SMA-negative MBs were also detected. These proliferating spindle mesenchymal cells were also positive for vimentin. No CD34 positive spindle cells were detected in MBs in this study. Then, these cells transformed into fibroblastic cells producing collagen fibers. MBs were negative for PTAH stain, when not contained fibrin. The MIB-1 stain disclosed the positive nuclei of epithelial cells on the surfaces of the MBs (Figure 2C-D, arrowheads) or the alveolar walls in the vicinity of the MBs. The many epithelial cells near the MBs were positive for SP-A, but not for SP-D, and the covering epithelial cells on the MBs were negative for SP-A.

DISCUSSION

Secondary cases were more frequent than primary cases in the OP pattern in pulmonary tissues, as shown in this study (Table 1). Although six primary cases were included in this study, histogenesis resulted in collagen vascular diseases (CVD), aspiration pneumonia, drug-induced cases, and one cancer-associated case (Table 1). Generally, OP patterns were also observed in acute infection or granuloma^[7]. Among primary IPs, the OP pattern is most often associated with NSIPs. Even if the case contained more than 70% of OP pattern in whole tissue, no statistically significant improvement was detected in the patient's prognosis^[8]. Although the authors of one study reported that the OP pattern of myxoid fibroplasia in DAD-OP may be associated with a better prognosis^[9], the OP pattern should

not generally be considered to be associated with a better prognosis in every case. In fact, OP pattern is very sensitive to steroid therapy^[10], very often recurs^[11,12] in the remaining fibrosis^[13], and gradually worsens^[14]. After endogenous or exogenous damage to the epithelial cells of AMs, the exudates including tissue exudates, inflammatory cells, and mesenchymal cells gradually organized into granulation tissues at the site of the AMs. If the stimulus to vascular walls was strong enough to allow the exudation of fibrin, positive PTAH staining was observed. Of course, in the present study, we have found the fibrin-positive MBs in AFOP case. Fibrin was stained dark blue in PTAH stain in this case (not shown). In this case, interstitial fibrosis was remained after disappearance of OP pattern. In particular, when fibrin exudation was observed to form fibrin balls and the patients needed a ventilator for repeated cases of OP, a condition known as AFOP, the prognosis was poor^[15]. However, some of these cases also resulted in a better prognosis^[15]. MBs were seemingly floating in the airspace, and only connecting with AMs. Along with the increase of MBs, epithelial damage of neighboring AMs might happen. In relapsing cases, a concentrically arranged myofibroblastic growth pattern was found in the MBs^[12]. These MBs are later fused with the alveolar septa at the point of the widening of the AMs.

As suggested, the initial stage of MB formation was the acute damage of the epithelial cells^[2]. We thought these epithelial cells should be those of the AMs, as demonstrated in the early stage of hyaline membrane formation^[1]. At the fine structural level, the basal lamina at the initial epithelial damage showed infoldings and deep invagination at this site, as reported^[2]. The airway MBs

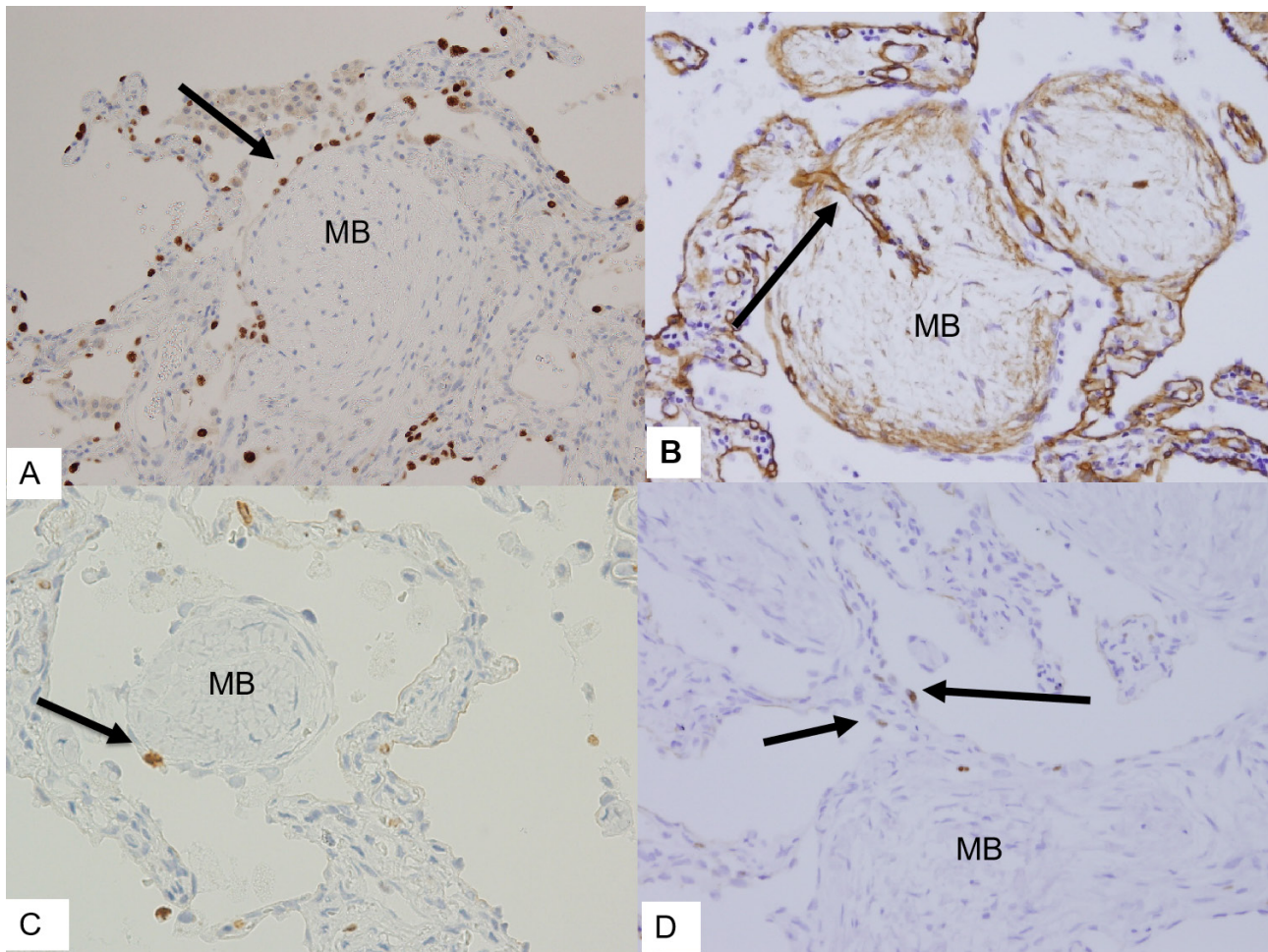


Figure 2 A: TTF-1-positive epithelium on MB. B: Collagen type IV-positive blood vessel (arrow). C: MIB-1-positive nucleus of the covering epithelium on MB (arrow). D: MB exhibiting MIB-1-positive nuclei (arrows) of the covering epithelia at the alveolar mouth. A-D: X200.

could then be capable of further damaging the other epithelia of neighboring AMs, resulting in connection with several AMs. Myofibroblastic transformation proceeded the epithelialization because myofibroblastic cells proliferated in the MBs, and often filled up the air space very rapidly, especially in the case of massive MB formation.

Blood vessels inside MBs extended from the original blood vessels of the alveolar septa through the AMs, as shown in Fig2B, probably owing to the effects of VEGF or bFGF^[4,5], which are secreted from the myofibroblasts of MBs, and their other role is the regeneration of the basal lamina^[9]. CD34 and collagen type IV antibodies clearly showed the extension of blood vessels through the AMs (Figure 2 B). The fibromyxoid matrix of the MBs appeared to possess many receptors for VEGF and bFGF; therefore, many blood vessels were present inside the MBs^[16].

Epithelial cells of MBs become positive for SP-A, but initially these covering epithelial cells are negative, owing to their immaturity. However, KL-6, which is a useful marker for the recognition of the activity level of interstitial pneumonia^[17,18], appeared in the very early stage on the covering epithelial cells. In fact, KL-6 initially appeared linearly on the epithelial cells. Therefore, KL-6 is very important for the morphogenesis of the lung as reported in fetal lung development^[19] and is also present in the stable phase of the lung^[20]. The maturation of epithelial cells happens after extension on MBs, and is associated with myofibroblastic cell proliferation inside MBs. At that point, the inflammatory cells and blood vessels were located in the center of the MBs. After the formation of MBs,

the fundamental pulmonary tissues were not destroyed, as revealed by elastic fiber staining, but later on, along with the widening of the AMs, which were connected to the MBs, internalization of the MBs proceeded gradually.

As demonstrated in this study, the covering epithelium of MBs was derived from the newly-divided alveolar epithelium associated with the maturational processes, and after completion of the maturational processes, they became positive for SP-A or SP-D on the surfaces of MBs as covering epithelial cells. These maturational processes will be further investigated.

Then, blood vessels within MBs were also supplied by the newly-formed ones through the AMs. MBs contained fibroblastic cells and myofibroblastic cells embedded in the matrix, as well as perivascular inflammatory cells, such as plasma cells (CD79a-positive), macrophages (CD68-positive), lymphocytes (CD20-positive) and a few eosinophils. These results were confirmed by immunohistochemistry, but not shown as figures.

CONCLUSIONS

MBs were connected with AMs, of which epithelium was to be considered the locus minoris resistentiae in the peripheral airways. Moreover, covering epithelial cells of MBs were newly formed alveolar epithelial cells immunopositive for AE1/AE3 (pancytokeratins), but negative for SP-A and SP-D. These epithelial cells of MBs were derived from the divided alveolar epithelium at AMs as shown MIB-1 (Ki-67)-positivity. MBs were fundamentally not contained elastic

fibers at all, that means no fundamental structural damage with MBs formation. But after diminishing MBs, some deformities have been induced because of fibrosis. KL-6 was expressed very early on the surface of these MBs. The blood supply of MBs happened through AMs by extension of the newly formed blood vessels as shown CD34-, Collagen Type IV- and Factor VIII related antigen-positivities.

Then, maturational processes of these newly-formed epithelium will be investigated in future. The examination of pathological changes of the lung, especially AMs of the alveolar ducts and alveolar sacs, may be a very important morphological checkpoint for the assessment of pulmonary function.

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