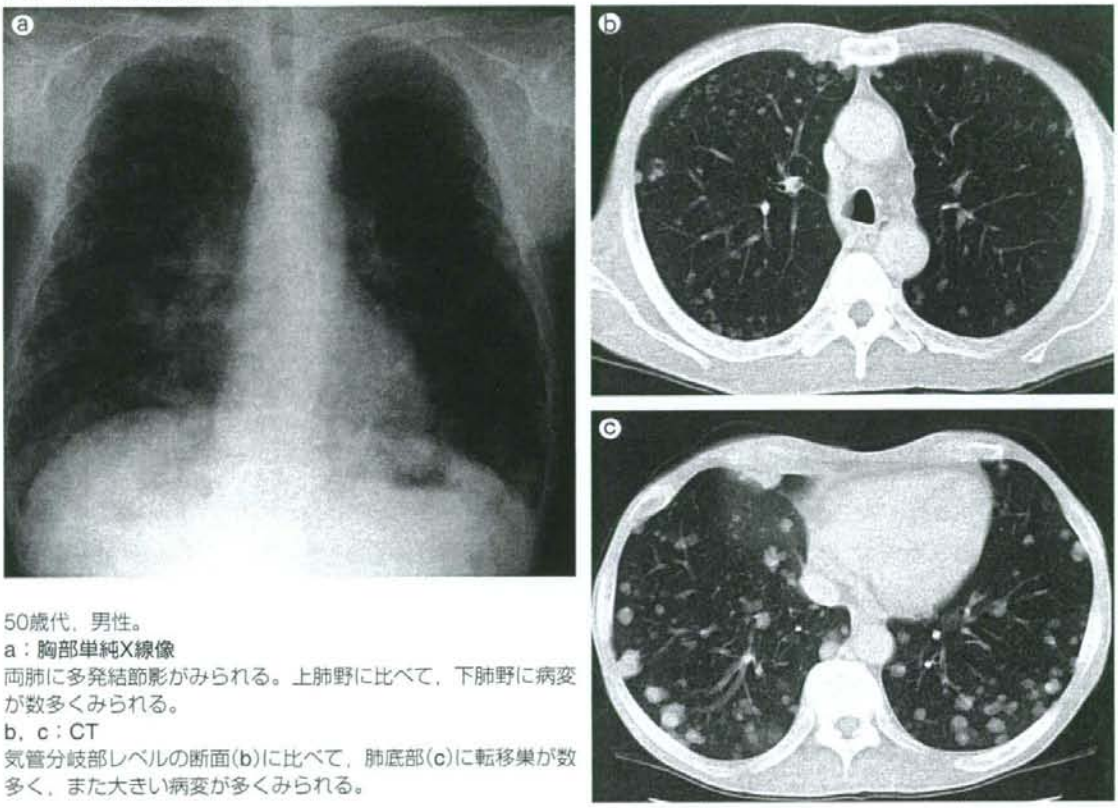
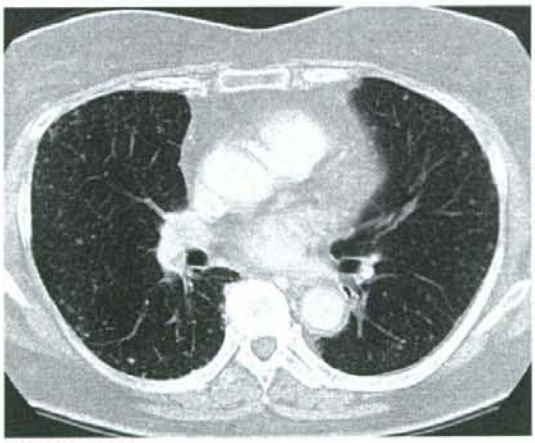
図2　腎盂癌の多発肺転移



50歳代，男性。
a：胸部単純X線像
両肺に多発結節影がみられる。上肺野に比べて，下肺野に病変が数多くみられる。
b，c：CT
気管分岐部レベルの断面(b)に比べて，肺底部(c)に転移巣が数多く，また大きい病変が多くみられる。

図3　膵臓癌の多発肺転移



70歳代，女性。
1cm再構成のCT
両肺に多数の粟粒状の小結節がみられる。膵臓癌の肺転移にしばしばみられる所見である。

が容易であるが，孤立性である場合，腫瘤が非常に小さい場合，辺縁不整である場合など，診断に難渋することもある。

画像所見のみからは原発巣を特定することは困難であることが多いが，数mmのびまん性の小結節影が散布し，粟粒結核との鑑別が問題となるような症例は，甲状腺癌，膵臓癌や卵巣癌の肺転移でみられる(図3)。逆に，非常に大きな腫瘤影で，いわゆる"cannon ball"タイプの転移は，大腸癌，腎癌，肉腫などでみられることが多いが，一概にはいえない(図4)。また悪性腫瘍の術後などに肺転移の出現を念頭に入れて定期的に経過観察を行っていれば，いきなり大きな肺腫瘤が見つかることは少ないはずである。

肺転移は辺縁平滑明瞭で充実性の円形影が基本であるが，辺縁が不規則となりノッチ形成を認めるような例やスピクラ様の所見がみられるものもある。ときに，大腸癌や乳癌の肺転移で

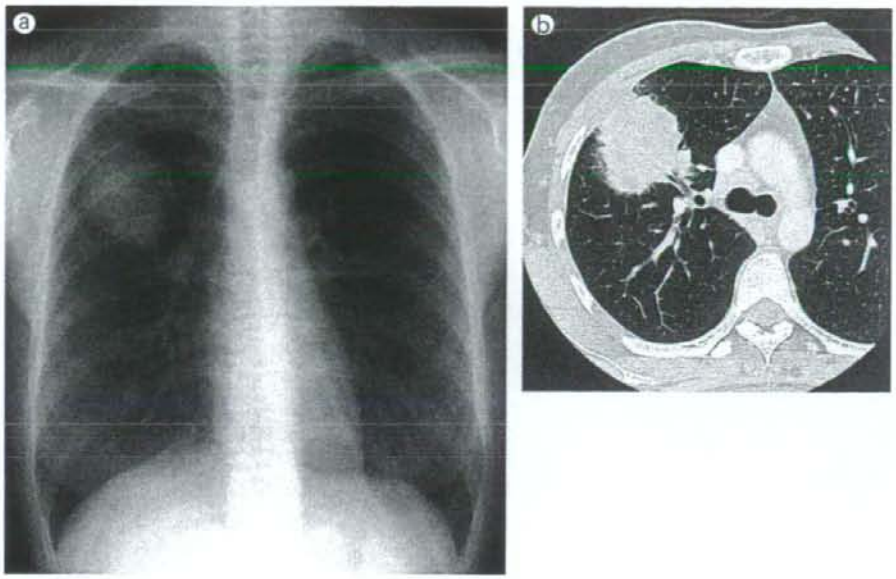
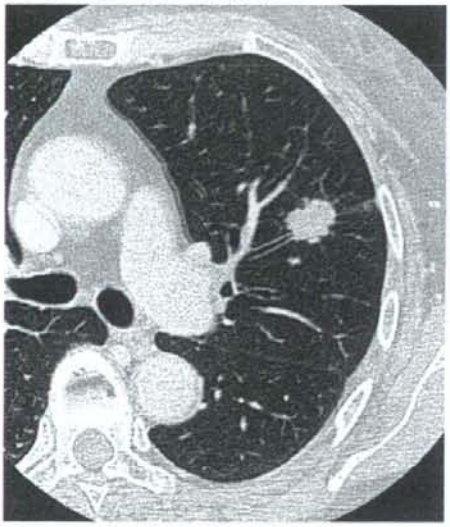


図4　大腸癌の孤立性肺転移

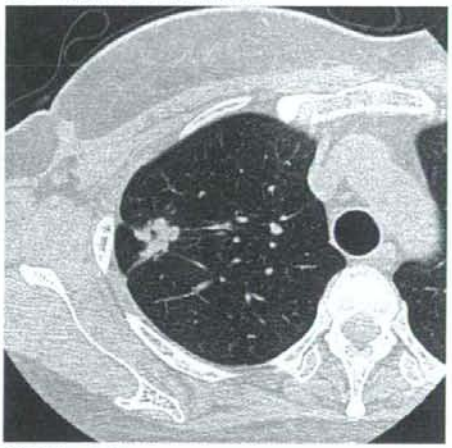
40歳代，女性。
a：単純X線像
b：高分解能CT
右肺上葉に大きな腫瘤影を1個認める。いわゆる"cannon ball"タイプの肺転移といえよう。大腸癌の術後であるが，術後に肺病変の定期的経過観察を受けていなかったために，発見時大きな腫瘤で見つかった。

図5　直腸癌の孤立性肺転移



70歳代，女性。
高分解能CT
左肺上葉に辺縁不整な結節を認める。辺縁にはスピクラがみられ，腫瘍による気管支の閉塞像もみられる。開胸生検によって直腸癌の転移と診断されたが，CT上は原発性肺癌との鑑別が困難である。

図6　乳癌の孤立性肺転移

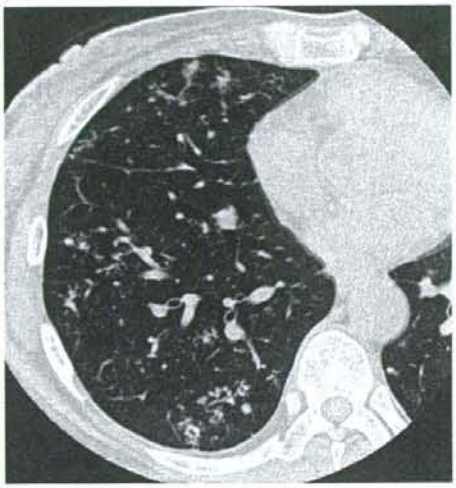


50歳代，女性。
高分解能CT
右肺上葉に辺縁不整な結節を認める。辺縁にはスピクラがみられ，腫瘤内にはair bronchogramもみられる。胸膜陥入像も明瞭である。開胸生検によって既往の乳癌の肺転移と診断されたが，CT上は原発性肺癌との鑑別が困難である。

みられ，孤立性の場合は原発性肺腺癌との鑑別が非常に困難である。術前の針生検や気管支鏡生検でも原発性肺癌との鑑別が困難なことがある[10]（図5，6）。

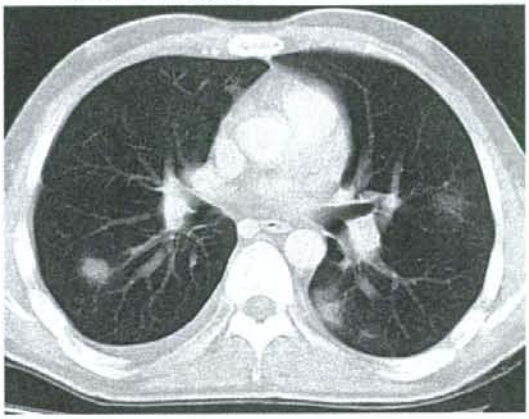
また，境界が不明瞭化するような肺転移としては，絨毛上皮腫や膵癌などが知られている（図7）。特に，結節や腫瘤の周囲に全周性にすりガラス影を伴う場合は，halo sign[*1]とよばれる。halo sign

図7　膵臓癌の多発肺転移



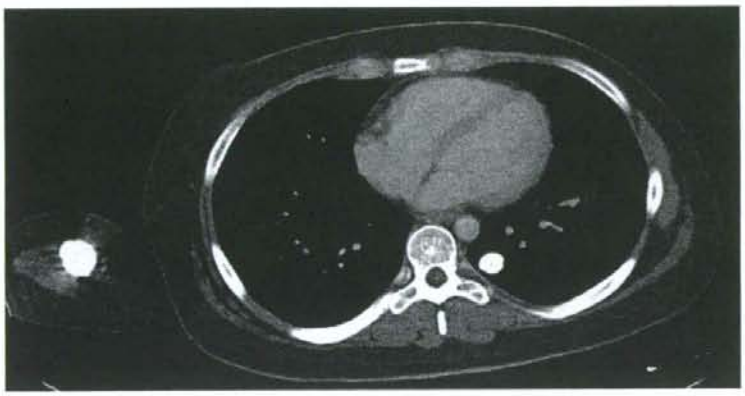
50歳代，女性。
高分解能CT
右肺に多数の小結節がみられる。小結節の辺縁は不明瞭で，一部すりガラス状にみえる部分もみられる。膵臓癌の肺転移にしばしばみられる所見である。

図8　絨毛癌の多発肺転移



30歳代，男性。
CT
両肺に多発結節がみられ，結節の周囲に全周性にすりガラス影を伴っている。原発巣は精巣で，絨毛癌の成分が9割の混合性胚細胞腫瘍であった。

図9　骨肉腫の肺転移
20歳代，女性。
CT
右上腕骨の骨肉腫の術後。左肺下葉に高度な石灰化を有する結節が出現した。切除により骨肉腫の肺転移が確認された。



はさまざまな疾患でみられるが，転移性肺腫瘍では，血管肉腫，Kaposi肉腫，絨毛癌などでみられることがある[11]（図8）。その他特徴のある肺転移として，石灰化を有する転移，空洞性肺転移，気管支内転移，癌性リンパ管症などがあり，これらについて概説する。

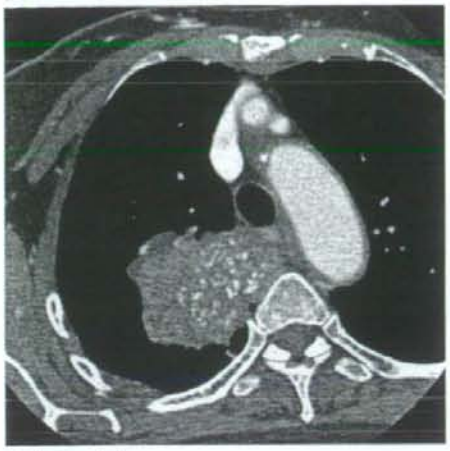
❋ 肺転移の石灰化

通常，肺結節内の石灰化の存在は良性結節であることが多く，画像診断上有用な所見である。しかし原発性肺癌や転移性肺癌のような悪性腫瘍であっても，まれではあるが石灰化を伴うことがあり，絶対的な鑑別点とはならない。悪性腫瘍内に石灰化がみられる場合，腫瘍そのものが石灰化を形成している

用語アラカルト

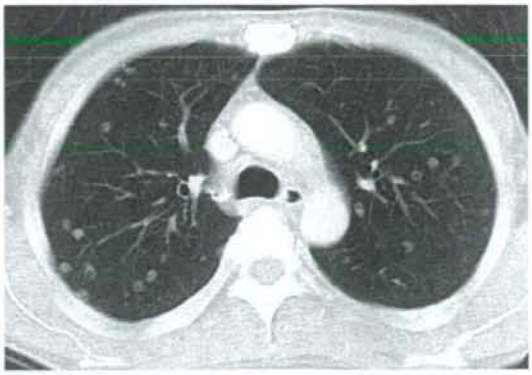
＊1 **halo sign**
CTにおいて結節の周囲にすりガラス状陰影が取り巻いている所見をさす。浸潤性肺アスペルギルス症において最初に記載され，出血性梗塞による陰影とされている。halo signは腫瘍の肺胞置換型浸潤や非出血性炎症性変化でもみられるが，腫瘍でみられる場合化学療法が著効して腫瘤が消退した際には，周囲のすりガラス状陰影も同時に消退する。

図10　大腸癌の肺転移



70歳代，女性。
CT
大腸癌術後で，右肺に多数の点状の石灰化を有する腫瘤がみられる。

図11　大腸癌の多発肺転移



50歳代，男性。
CT
両肺に多発する小結節を認める。一部の結節に空洞がみられる。

場合と，元来陳旧性肺結核などで肺内に石灰化があり，その部位に腫瘍が進展したために，結果的に腫瘍内に石灰化がみられる場合がある。

石灰化を示す転移性肺腫瘍の代表的疾患としては，古くより骨肉腫の肺転移が知られている(図9)。この場合の石灰化の機序はosteoid matrix内の骨化によるとされている。大腸癌の肝転移がしばしば石灰化を有することは知られているが，肺転移でも点状の石灰化をみることがある[10]（図10)。

※ 空洞形成

転移性肺腫瘍の空洞形成は比較的少ないとされるが，まれなものではない。原発巣としては頭頸部などを原発とする扁平上皮癌が最も頻度が高く，ときに膀胱腫瘍などの移行上皮癌や大腸癌でもみられる(図11)。空洞形成の機序としては，腫瘍内壊死が気管支と交通して気道内に壊死物質が排泄される結果とされている。ときに腫瘍壊死は気管支胸膜瘻を生じることがあり，その結果気胸が合併する場合がある。空洞形成の別のメカニズムとしては，腫瘍による細気管支閉塞のためチェックバルブ機構によって末梢に嚢胞が形成されるような場合もある。肺に薄壁空洞の多発性転移をきたし，気胸を合併しやすい腫瘍として，皮膚の血管肉腫がよく知られている[12]。

※ 気管支内転移

転移性腫瘍が中枢気管支を閉塞し，肺門部肺癌と臨床的に類似した病態を示すいわゆる気管支内転移は，比較的まれではあるが，腎癌，乳癌，大腸癌，悪性黒色腫などでみられる。画像所見としては，気管支閉塞に伴う二次性変化として無気肺，閉塞性肺炎像を示すが，転移の初期に画像で捉えられると，二次性変化がみられず腫瘍そのものがみられることもある。画像的には肺門部肺癌との鑑別は困難であり，診断には気管支鏡による直視下生検が必要となる。

※ 癌性リンパ管症

癌性リンパ管症は，多くは肺末梢の血行性肺転移からはじまり，末梢のリンパ管を浸潤し，肺門へと進展するのが一般的な経路といわれるが，一部で肺門リンパ節から逆行性に進展した症例もあると考えられている。胃癌，乳癌で多くみられ，原発性肺癌でも癌性リンパ管症の進展を示すものがみられる。

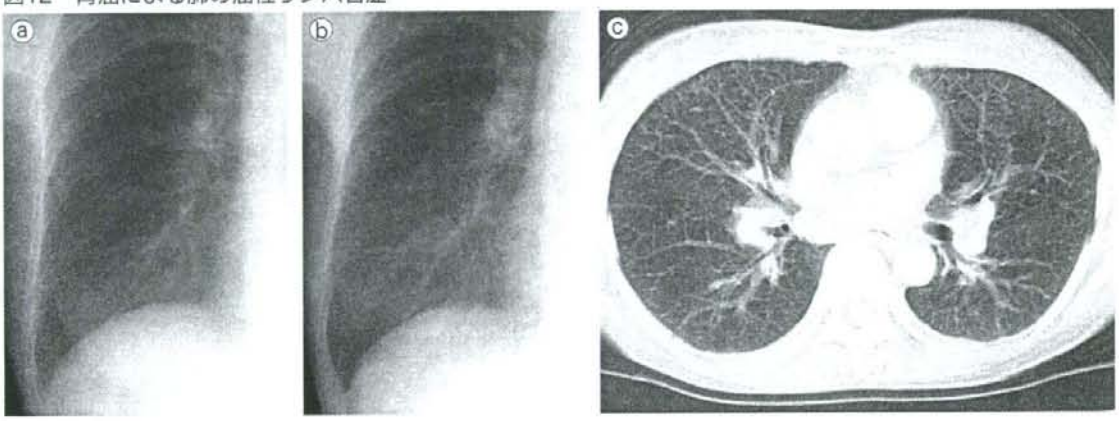
胸部単純X線写真では典型的には血管，気管支周囲の間質性変化を反映して，肺門より末梢に広がる線状影や，小葉間隔壁の肥厚を反映してKerley's B-lineが認められる。

胃癌や乳癌では両側性であることが多いが，肺

癌症例などでは片側性であることが多い。1枚のX線写真ではその変化が微妙であることが少なくなく，以前のフィルムとの比較が重要である（図12）。ときに呼吸困難の症状が強いにもかかわらず，胸部X線所見で異常の指摘が困難な場合もあるが，その場合でもCTによって異常を検出できることが多い。

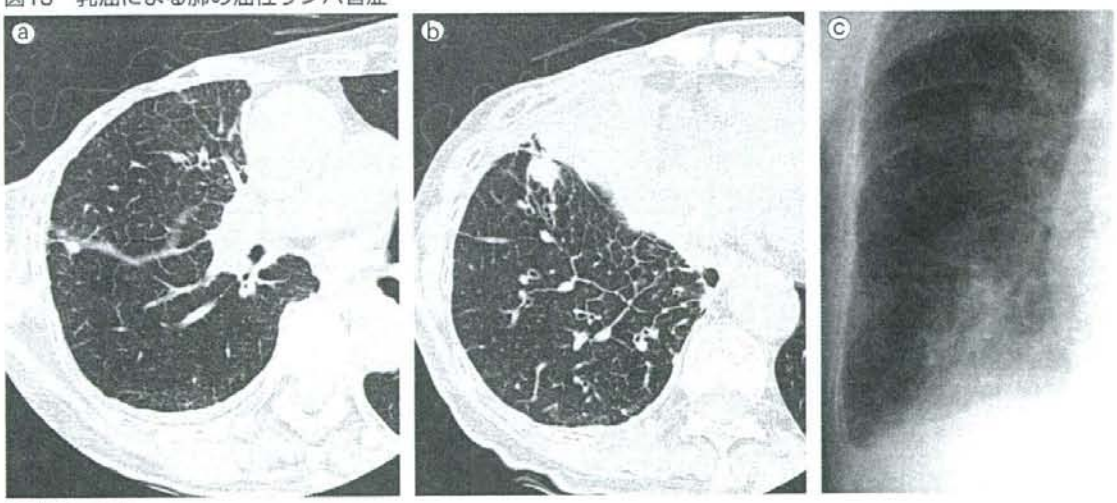
CT，特に高分解能CTはほかのびまん性肺疾患との鑑別に有用で，その所見としては，気管支血管周囲間質の不規則な肥厚，小葉間隔壁の肥厚がある。この所見は癌性リンパ管症に比較的特徴的である（図13）。癌性リンパ管症の診断には高分

図12　胃癌による肺の癌性リンパ管症



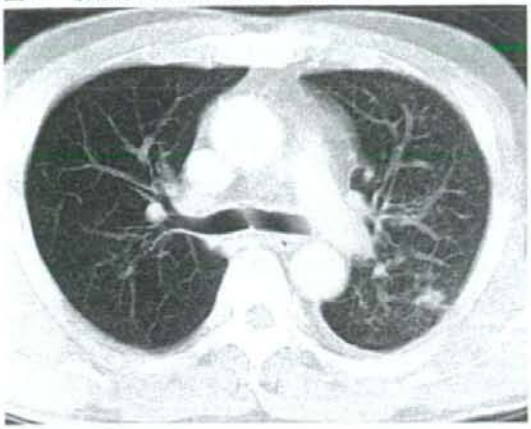
70歳代，女性。
a：発症前の胸部X線写真，b：癌性リンパ管症発症後の胸部X線写真
右下肺野に発症前ではみられなかった多発粒状影が出現している。軽微ではあるが，Kerley's B-lineも出現している。
c：1cm再構成のCT
両側肺末梢側に線状影や小葉間隔壁の肥厚像がみられ，肺野全体がすりガラス状である。両側肺門および縦隔リンパ節腫大もみられる。

図13　乳癌による肺の癌性リンパ管症



60歳代，女性。
a，b：高分解能CT
右肺下葉に小葉間隔壁の肥厚像がみられる。小葉間隔壁の肥厚像は網目状で，ところによっては不整像もみられる。これらの線状影は胸膜に連続している。
c：CT撮影時よりやや進行した時点での単純X線像
胸膜から直角にみられる短い線状影Kerley's B-lineが明瞭にみられている。

図14　肺癌による癌性リンパ管症



60歳代，男性。
左上葉原発の肺癌症例の1cm再構成のCT
左肺は右肺に比べて全体にすりガラス状で，細かい線状影がやや目立つ。左側の気管支壁の肥厚像もみられる。左肺全体に癌性リンパ管症が広がっていると考えられる。

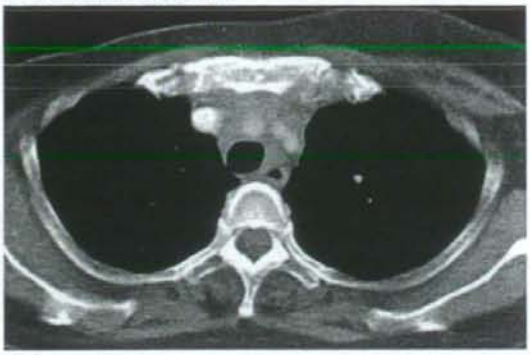
解能CTが有用であるが，1cm厚で再構成されたCTでも細かい線状影やすりガラス状影，気管支血管束の肥厚像などに着目すると診断可能なこともある(図14)。

癌性リンパ管症の場合，CT上気管支周囲間質の肥厚のみが目立って観察される症例に比べて，小葉間隔壁の肥厚が目立って観察される場合のほうが肺機能の障害の程度が強い[13]。癌性リンパ管症は通常予後が悪いが，化学療法の進歩で比較的長期生存例や癌性リンパ管症が画像消失する例も報告されるようになってきた[14] (Pitfall参照)。

縦隔，肺門リンパ節転移

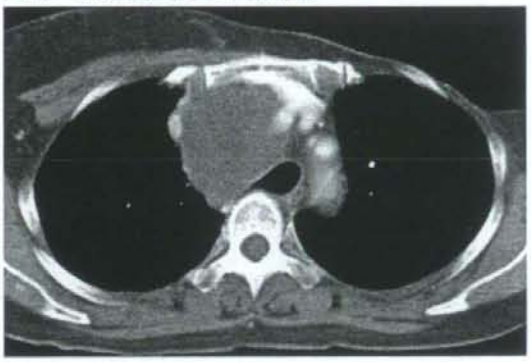
縦隔や肺門のリンパ節に転移をきたす悪性疾患は肺癌が圧倒的に多く，食道癌でも縦隔リンパ節転移がしばしばみられる。それ以外で縦隔リンパ節転移をきたす悪性疾患として乳癌，頭頸部癌，

図15　乳癌の縦隔進展



50歳代，女性。
CT
縦隔内の大血管周囲にみられる脂肪を置き換えるように軟部組織濃度域がみられる。乳癌の縦隔進展と考えられる。

図16　乳癌の縦隔リンパ節転移



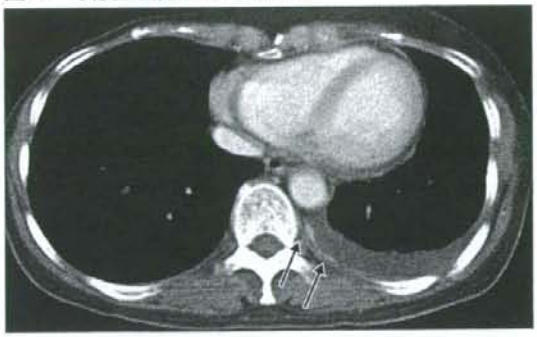
60歳代，女性。
CT
左乳癌術後。気管前のリンパ節が高度に腫大している。針生検で乳癌の転移が確認された。

睾丸腫瘍などがある。乳癌は胸骨あるいは傍胸骨領域から縦隔に直接浸潤する場合がある。縦隔リンパ節が累々と腫大するような転移形式もあるが，縦隔内に直接浸潤した場合は縦隔内の脂肪を置き換えるように進展する(図15, 16)。また肺癌以外で，肺門部リンパ節転移をきたすことはまれである。

Pitfall

- 癌性リンパ管症の診断は，高分解能CTが撮影されている場合で典型像を示せば比較的容易である。しかし肺癌の経過観察や悪性腫瘍の肺転移検索に撮影される胸部CTは通常の1cm厚で再構成されている場合が多く，癌性リンパ管症が比較的軽微な場合，その存在を見落としてしまうことがありうる。
- 1cm再構成の通常CTでの癌性リンパ管症の診断のポイントは，小葉間隔壁の肥厚を反映した微細線状影や気管支血管束の肥厚に加え，非区域性のすりガラス状にみえる陰影を注意すべきである。
- 1cm再構成のCTでも健常部の肺や過去のCTと比較することで，癌性リンパ管症の存在を見落とさずに推定できることが多い。

図17　右乳癌術後の左胸膜転移



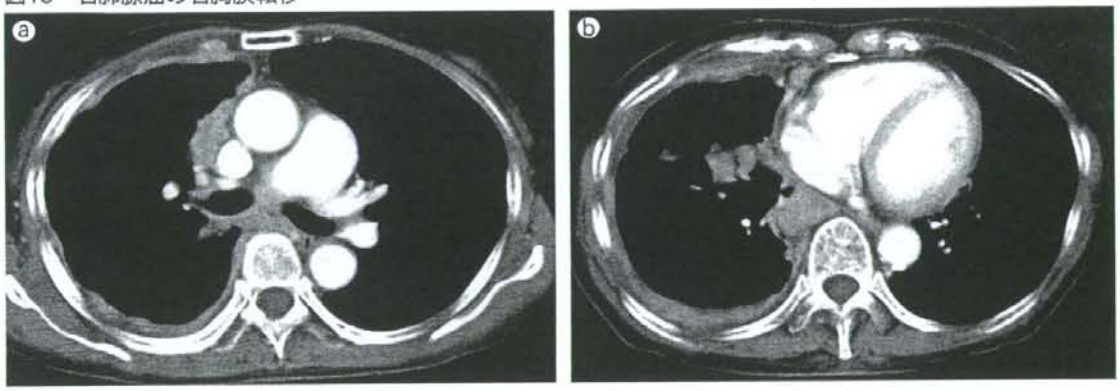
40歳代，女性。
造影CT
右乳癌術後。左胸水貯留がみられ，胸水と胸壁の間によく造影される不整な線状構造がみられる(↑)。胸水貯留を伴った胸膜転移であるが，造影CTでその存在と範囲が明瞭になる。

胸膜転移

胸膜は，肺やリンパ節と比べて悪性腫瘍の転移の起こりやすい部位ではないが，ときに胸膜転移をみることがある。CT所見としては，胸膜の不整肥厚像，限局性結節状あるいは肥厚像などである。肺野条件で肺側への凸な腫瘤をみれば胸膜転移の可能性がある。

一方，胸膜に沿った限局性の肥厚が散在性，両側性にみられ，特に石灰化を伴っている場合はアスベスト曝露による胸膜プラーク*2であることが

図18　右肺腺癌の右胸膜転移



60歳代，女性。
a，b：造影CT
右胸膜がびまん性かつ不整に肥厚しており，造影CTでよく造影されている(a，b)。胸膜生検で腺癌との病理診断が得られた。右肺下葉の縦隔側の腫瘤が原発巣かと推定されるが，断定困難である(b)。画像所見のみからは，悪性胸膜中皮腫との鑑別が難しい。

用語アラカルト

＊2 胸膜プラーク
アスベストの低濃度曝露で多くみられる所見である。胸膜斑ともよばれる。限局性のヒアリン化線維性胸膜病変で，しばしば石灰化を伴う。好発部位は，横隔膜直上部，下葉や中葉の壁側面などで，肺尖部や肋横隔膜部には通常みられない。CTでは平板状，台形状の肥厚像を示し，石灰化が容易に捉えられる。

Tips & Tips

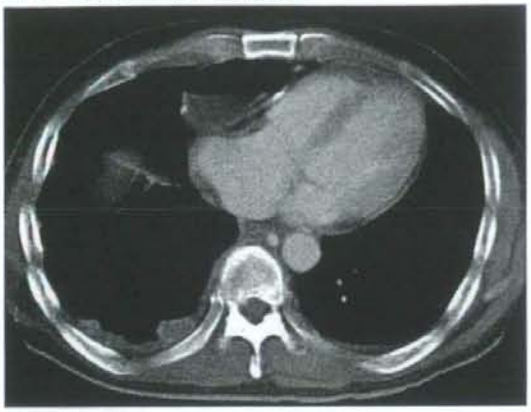
・画像診断で悪性腫瘍をより考える所見，良性病変をより考える所見は数多くみられるが決定的なものはない，といっても過言ではない。しかし「胸膜肥厚が胸壁側胸膜だけでなく縦隔側胸膜にみられた場合は悪性病変を示唆する」という所見はかなり信頼精度が高く，臨床上「使える」所見である，というのが筆者の印象である。
・感染性の胸膜炎，膿胸，アスベスト曝露による胸膜プラークなどでは多くの場合，胸壁側の胸膜の肥厚に留まり，縦隔側の胸膜に肥厚がみられることはまれである。また胸膜プラークは横隔膜面の胸膜にはむしろ多くみられる。

多いので注意を要する。胸水貯留がみられると胸膜病変の診断は難しくなるが，造影CTを行うと胸膜転移は比較的よく造影されて胸水や胸壁から識別できることが多い(図17)。また胸膜の肥厚部位については，胸壁側胸膜だけでなく縦隔側胸膜に肥厚がみられた場合は悪性病変を示唆する所見とされている[15] (Tips参照)。

胸膜転移をきたす疾患は肺癌が最も多いが，肺癌の場合は進行すると胸膜転移なのか胸膜への直接浸潤なのかよくわからない場合もある。特に肺腺癌で胸膜に沿って悪性中皮腫様に進展するものがあり，この場合画像上では悪性中皮腫との鑑別がきわめて困難となる(図18)。

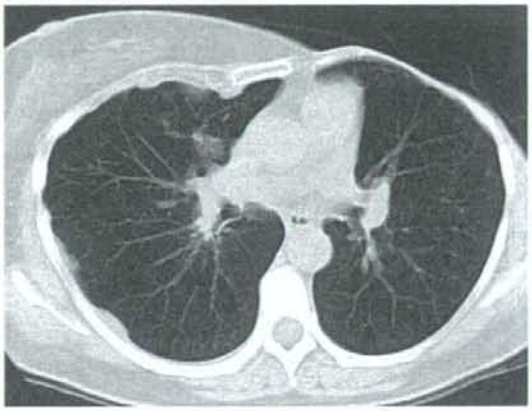
肺癌以外の疾患では，胸腺腫にしばしば胸膜播種をきたすことが知られており，食道癌や乳癌などにも胸膜転移がみられることがある(図19)。乳癌の場合は，乳癌の病巣の同側の胸膜に転移が起こりやすいというわけでなく，反対側にでも胸膜転移は起こる(図20)。胸郭外の腫瘍からの胸膜転移はまれであるが，腎細胞癌で胸膜転移がみられることがある(図21)。

図19　食道癌術後の右胸膜転移



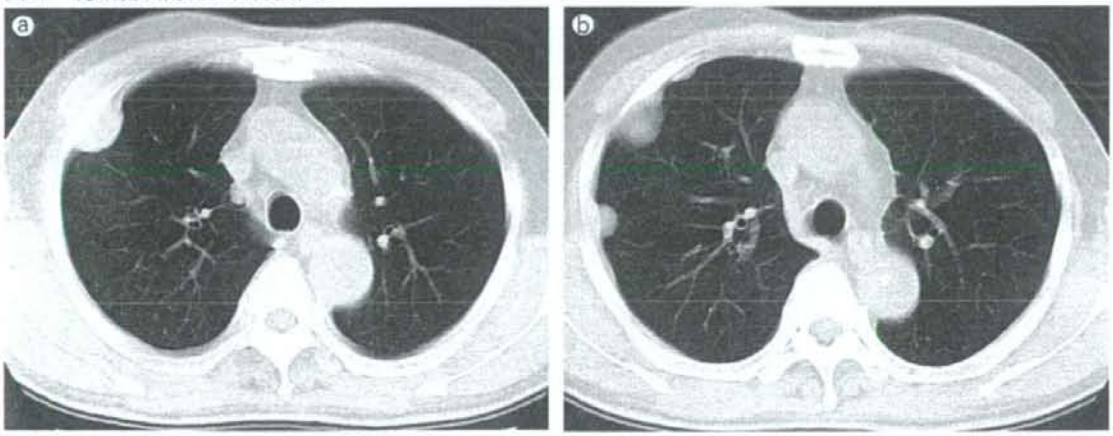
40歳代，男性。
造影CT
右胸膜の背側部が結節状に肥厚しており，造影CTでよく造影されている。

図20　左乳癌の右胸膜転移



40歳代，女性。
CT
左乳癌術後。肺野条件で乳癌とは反対側の右胸壁側の胸膜が結節状に肥厚している。

図21　腎細胞癌術後の右胸膜転移



80歳代，男性。
a，b：CT
右胸膜上に，extrapleural sign陽性の腫瘤が多発してみられている。

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診療の秘訣

アスベストによる胸膜中皮腫早期病変を見逃さないために

岸本　卓巳
岡山労災病院副院長・内科

アスベストによって発生する中皮腫が社会問題化している．中皮腫の80%以上を占める胸膜中皮腫は呼吸困難や胸痛を主訴として来院し，確定診断される場合が多い．しかし，自覚症状で来院した場合にはほとんどが進行期病変である．われわれが平成17年度から行った中皮腫の過去症例の追跡調査では，自覚症状がなく健康診断で胸水を指摘され，経過観察されていたり，結核性胸膜炎として治療されている間に画像上典型的な胸膜中皮腫像を呈したため，確定診断に至った症例が少なくないことが判明した．また，74%には職業性石綿ばく露を認めており，日本の中皮腫も石綿ばく露によって発生していることを確認した．

現時点では，胸膜中皮腫の有効な治療方法がなく，早期病変をきちんと診断して胸膜肺全摘出術を行うことが中皮腫の予後を改善する唯一の方法である．

胸部画像上，胸水を呈する患者を診療する際に注意することは，アスベストを直接あるいは間接的に職業上で使用したことがあるかどうか，また，家庭や環境でばく露した形跡がないかなどについて詳細な問診を行うことである．また，必ず胸部CTを撮影し，アスベストによる胸膜プラークの存在の有無を確認する．たとえ，腫瘍性胸膜肥厚や腫瘤がなくても，縦隔側胸膜の肥厚像は中皮腫の比較的早期病変である可能性があるので，必ず確認しておく（図）．

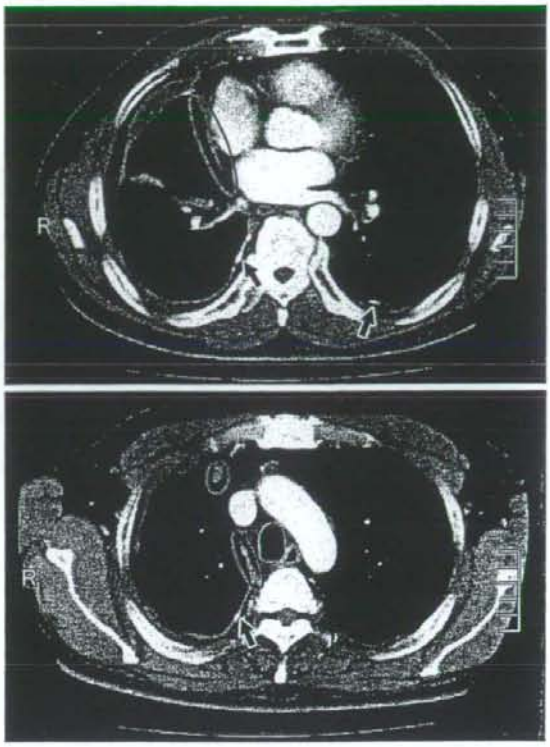

図
右縦隔側の胸膜肥厚像（赤丸印）が，中皮腫による変化である．この部位には脂肪層が認められるのみであるから，石灰化胸膜プラーク（黒矢印）が認められるような症例の場合，この程度の肥厚でも胸膜中皮腫を疑うべきである

胸水が穿刺によって検査ができる程度に貯留している場合には，試験穿刺を行って，その性状とともに，CEA，ADA，CYFRA21-1，ヒアルロン酸の測定と細胞診を行っておく．細胞診はパパニコロウ染色のみならず，cell peletsを作成し，カルレチニン，CEAなどを使用した免疫染色も行っておく．細胞診で胸膜中皮腫を診断できる可能性は40%以下であることを十分認識しておく．胸水中CEAが低値で，ヒアルロン酸が10万ng/ml以上あるいはCYFRA21-1が50 ng/ml以上の場合には，胸部画像上腫瘍性胸膜肥厚が認められない場合でも，胸腔鏡を行い壁側胸膜の観察と生検を行っておくべきである．生検はできるだけ大きく，深く採取することが確定診断上重要である．隆起性病変を認めない場合には，胸膜下を腫瘍が進展する肥厚型である場合もあるので，胸壁が透けて見えない場合にはその部位は必ず生検

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しておく．万一，中皮腫であるとは診断できなかった場合にも，原因が不明で，職業性石綿ばく露がある場合には良性石綿胸水と診断される場合もあり，労災補償の対象となる場合には申請を行う．

胸水がわずかで，穿刺できないときは経過を慎重に追うことが重要であり，増加する場合には胸膜中皮腫の可能性も念頭においておく．

Immunological Changes in Mesothelioma Patients and Their Experimental Detection

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Abstract: It is common knowledge that asbestos exposure causes asbestos-related diseases such as asbestosis, lung cancer and malignant mesothelioma (MM) not only in people who have handled asbestos in the work environment, but also in residents living near factories that handle asbestos. These facts have been an enormous medical and social problem in Japan since the summer of 2005. We focused on the immunological effects of asbestos and silica on the human immune system. In this brief review, we present immunological changes in patients with MM and outline their experimental detection. For example, there is over-expression of *bcl-2* in CD4+ peripheral T-cells, high plasma concentrations of interleukin (IL)-10 and transforming growth factor (TGF)-ß, and multiple over-representation of T cell receptor (TcR)-Vß in peripheral CD3+ T-cells found in MM patients. We also detail an experimental long-term exposure T-cell model. Analysis of the immunological effects of asbestos may help our understanding of the biological effects of asbestos.

Keywords: asbestos, immunology, mesothelioma, chrysotile

Introduction

It is common knowledge that asbestos exposure causes asbestos-related diseases such as asbestosis, lung cancer and malignant mesothelioma (MM) not only in people who have handled asbestos in the work environment, but also in residents living near factories that handle asbestos. These facts have been an enormous medical and social problem in Japan since the summer of 2005 (Kanazawa et al. 2006; Murayama et al. 2006; Nakano, 2006). Several patients with MM living in Amagasaki, Hyogo prefecture, Japan have featured in news reports. These patients resided 1 km from an asbestos factory and had no identifiable occupational exposure to asbestos. Given that MM is an incurable disease and prognosis is not promising (Vogelzang and Pass, 2006; Zucali and Giaccone, 2006; Tsiouris and Walesby, 2007), and considering the absence of effective government legislation concerning the usage of asbestos, people in Japan have become concerned about social and medical issues related to asbestos.

Asbestos is categorized as a silicate (mineralogical complexes containing metals, such as iron and magnesium) and includes forms such as chrysotile, crocidolite, and amosite. Patients exposed to asbestos develop pulmonary fibrosis known as asbestosis, mesothelial plaque and malignant diseases such as lung cancer and MM (Niklinski et al. 2004; Becklake et al. 2007; O'Reilly et al. 2007). The mechanisms of asbestos-induced carcinogenesis are thought to produce an accumulation of DNA damage due to asbestos-induced production of reactive oxygen/nitrogen species (ROS/RNS) and an escape from the asbestos-induced activation of the mitochondrial apoptotic pathway (Shukla et al. 2003; Upadhyay and Kamp, 2003). In addition, we believe that some of these malignancies may be caused by a decline in tumor immunity owing to exposure of immunocompetent cells to asbestos.

Silica is known as one of the strongest environmental substances that cause autoimmunity dysfunction (Hess, 2002; Cooper and Parks, 2004). Silicosis patients often develop immunological complications such as rheumatic arthritis (known as Caplan syndrome (Caplan, 1953)), systemic sclerosis (SSc), and systemic lupus erythematoses (SLE). The effects of silica on autoimmunity have also been recognized

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following the discovery that patients who receive plastic surgery with implants containing silicone ($[SiO_2\text{-}O\text{-}]_n$) show frequent complications involving autoimmune disorders (Shons and Schubert, 1992; Hirmand et al. 1993). These findings clearly indicate that crystalline silica causes dysregulation and/or disturbance of the human immune system, particularly autoimmunity.

The overall evidence suggests that asbestos may influence human immunocompetent cells and that such alterations may affect the occurrence and progression of asbestos-related malignant diseases. Thus, we have focused on the immunological effects of asbestos. Among the many types of asbestos, chrysotile has mainly been used in our experiments. It is known that magnesium, the main compartment of chrysotile as silicate, usually dissociates from the chrysotile core (SiO_2) in the human body after inhalation, and chrysotile is known to induce malignant transformation. However, its carcinogenic capacity is lower than that of other forms of iron-containing asbestos such as crocidolite and amosite (Harington, 1991).

In this article, we present immunological changes in MM patients with our experimental model. These changes may have resulted from the immunological effects of asbestos on human immunocompetent cells, and may offer some suggestions for the immunological prevention of the occurrence and progression of asbestos-induced malignant diseases.

bcl-2 expression of peripheral CD4+ T cells in MM patients

As shown in the upper panel of Figure 1-A, peripheral CD4+ T cells from MM patients showed a significantly higher expression of *bcl-2* compared to that of healthy volunteers (Miura et al. 2006). This may suggest that the over-expression of *bcl-2* in peripheral CD4+ T cells is one of the markers for the occurrence of MM, although it should be determined whether many cancer-bearing patients respond in a similar manner. The experimental background of this finding is as follows.

Experiments that exposed a high dose of chrysotile to peripheral fresh T cells or T cell-derived cell lines for a short time revealed that a human T-cell leukemia virus type-1 (HTLV-1)-immortalized human polyclonal T-cell line, MT-2, underwent apoptosis with ROS production via activation of the mitochondrial apoptotic pathway with the phosphorylation of p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK) signaling molecules. In addition, we observed a shift of the Bax-dominant Bax/Bcl-2 balance, the release of cytochrome-c from mitochondria into the cytosol, and the activation of caspases 9 and 3 upon short-term, high-level exposure to chrysotile (Hyodoh et al. 2005). However, we thought that an *in vitro* experimental model of chronic exposure was necessary in order to analyze the immunobiological effects of silicates during long-term exposure and to transfer these experimental findings to clinical analyses.

Thus, we established a chrysotile-induced apoptosis-resistant subline of MT-2 (MT-2Rst), and characterized the cell biological differences between the original MT-2 cell line (MT-2Org) and MT-2Rst. MT-2Rst cells were characterized by (i) an enhanced expression of *bcl-2* as shown in the lower panel of Figure 1-A, restoring apoptosis sensitivity with the decrease in *bcl-2* expression level by siRNA, (ii) excessive interleukin (IL)-10 secretion and expression, and (iii) the activation of signal transducers and activators of transcription (STAT) 3 inhibited by 4-amino-5-(4-chlorophenyl)-7-(t-butyl) pyrazolol [3,4-d] pyrimidine (PP2), a specific inhibitor of Src family kinases. These findings suggest that contact between cells and asbestos may affect the human immune system and trigger a cascade of biological events, such as the activation of Src family kinases, enhancement of IL-10 expression, STAT3 activation, and Bcl-2 over-expression as previously reported (Miura et al. 2006).

Another interesting finding was obtained from analyses using *bcl-2* expression in peripheral CD4+ T cells. We performed factor analysis using various clinical parameters and the *bcl-2* relative expression ratio (*bcl-2* RER) obtained by real-time RT-PCR from MM patients. Our results revealed that *bcl-2* RER, a past history of asbestos exposure, peripheral platelet counts, and serum CRP values formed one factor, and these parameters exhibited higher, present, lower count, and lower values, respectively, as shown in Table 1. Platelet-derived growth factor (PDGF) is one of the widely known MM-related growth factors and it functions as the autocrine/paracrine proliferation-promoting factor for mesothelioma cancer cells (Langerak et al. 1996, Klominek et al. 1998). Although higher serum levels of PDGF in MM patients are thought to be produced from mesothelioma cells and *bcl-2*

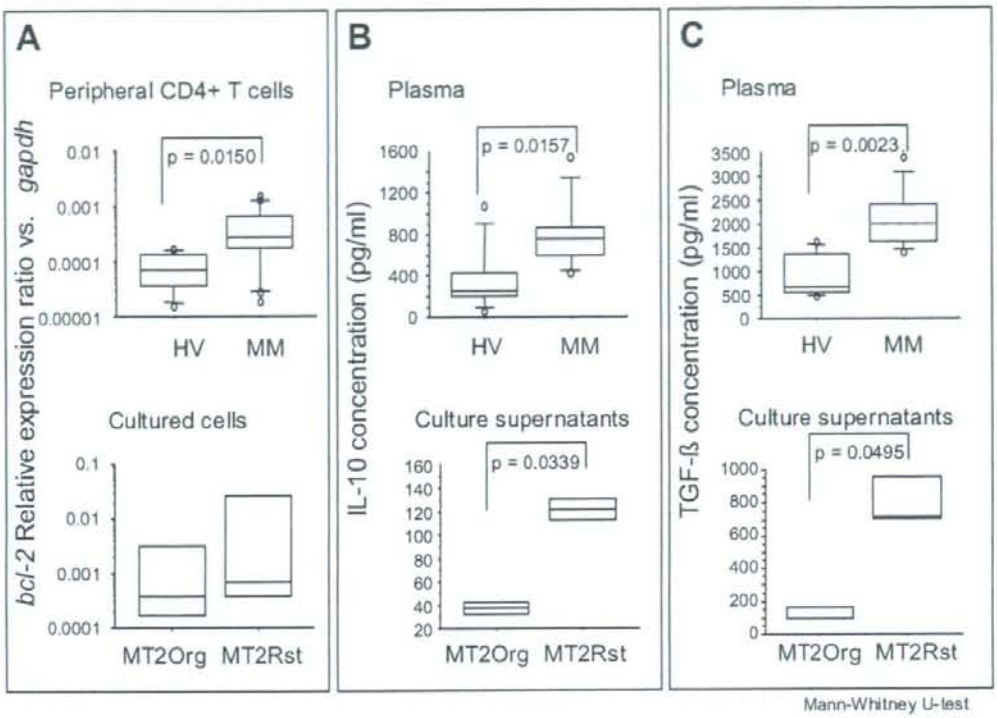

Figure 1. Comparison of *bcl-2* relative expression ratio vs. *gapdh* plasma concentrations in anti-inflammatory cytokines in MM patients and healthy volunteers (HV), and *bcl-2* expression and secretion of these cytokines from experimental low-dose and long-term exposed T-cell models to asbestos (MT-2Org and MT-2Rst, see text for details).

Panel **A** shows the relative expression ratio of *bcl-2* in peripheral blood CD4+ cells (upper panel) from MM patients and HV, or in cultured MT-2Org and MT-2Rst cells (lower panel). Panels B and C show the plasma concentrations of IL-10 (**B**) and TGF-ß (**C**) from MM patients and HV (upper panels), or the concentrations in culture supernatants of IL-10 (**B**) and TGF-ß (**C**) from MT-2Org and MT-2Rst cells (lower panels).

Peripheral blood mononuclear cells (PBMCs) were isolated from the heparinized blood of healthy donors and MM patients using a Ficoll-Hypaque density gradient (Separate-L®, Muto Pure Chemicals Co. Ltd., Tokyo, Japan). For the isolation of CD4+ T cells, PBMCs were further separated using Magnetic Cell Separation (MACS) CD4 MicroBeads (Miltenyi Biotech, Bergisch Gladbach, Germany) according to the manufacturer's instructions. The enriched cells were >90% pure as determined by flow cytometry. Specimens were taken from healthy volunteers and patients from whom informed consent had been obtained. The Institutional Ethics Committee of Kawasaki Medical School, Hyogo College of Medicine, and Okayama Rosai Hospital approved the project. A fluorescence thermocycler (Mx3000P® QPCR System, Stratagene Corporation, La Jolla, CA) was used for real-time RT-PCR experiments by following the instructions of the manufacturer. The fluorescence-labeled amplification product is measured continuously with this technique. Total RNA obtained from CD4+ T cells isolated from peripheral CD4+ T cells was extracted using an RNA Bee kit (Tel-Test, Inc., Friendswood, Texas), and 5 µg of RNA was reverse-transcribed with standard methods using a RevertAid™ H Minus First Strand cDNA Synthesis Kit (Fermentas International Inc., Ontario, Canada). An amount of cDNA equivalent to 50 ng of RNA served as the template for PCR in a volume of 20 µl (each primer and SYBER Premix Ex Taq, TaKaRa). The primers for *bcl-2* and *gapdh* were added to the same reaction tube at the optimal concentration for each primer set and PCR was performed. Primers were as follows: *bcl-2*; 5'-TGATGTGAGTCTGGGCTGAG-3' (Forward: Fw) and 5'-GAACGCTTTGTCCAGAGGAG-3' (Reverse: Rv), Bax; 5'-AGTAACATGGAGCTGCAGAGG-3' (Fw) and 5'-ATGGTTCTGATCAGTTCCGG-3' (Rv), *gapdh*; 5'-GAGTCAACGGATTTGGTCGT-3' (Fw) and 5'-TTGATTTTGGAGGGATCTCG-3' (Rv).

The relative expression of various target genes such as *bcl-2* was calculated as follows when real-time RT-PCR was performed: [A: number of PCR cycles required to reach a certain intensity of fluorescence for the *gapdh* product. B: number of PCR cycles required to reach the same fluorescent intensity for the target gene product (*bcl-2*) derived from the same sample.] The relative level of the target gene is expressed as 1/2[B-A], with gapdh expression being 1.0. PCR products were confirmed to be successfully amplified by standard agarose gel electrophoresis and staining with ethidium bromide. Comparisons of the results for relative gene expression and proliferation assayed by real-time RT-PCR were analyzed using the Mann-Whitney U-test.

Cytokines in plasma from MM patients and HV and culture supernatant were measured using an ELISA kit (Quantikine® Human TGF-ß1 (or IL-10) Immunoassay; R&D Systems) and the Cytometric Bead Array of Human Th1/Th2 cytokine kit II (CBA, BD Bioscience, San Jose, CA, U.S.A.), and measurements were made using FACSCalibur flow-cytometry (BD Bioscience) according to the manufacturer's instructions.

Table 1. Factor analysis of clinical parameters in mesothelioma patients with relative *bcl-2* expression in peripheral CD4+ T cells.

Parameter	Value (a value of more than ± 0.4 is thought to contribute to the formation of this factor)
bcl-2 relative expression ratio in peripheral CD4+ T cells	**0.59009**
Histology (numbered) epithelial type = 1 mixed type = 2 sarcomatous type = 3	–0.14234
Past asbestos exposure (numbered) existence = 1 unknown = 2 none = 3	**0.55496**
White Blood Cell count	0.22054
Platelet count	**–0.76064**
Concentration of serum creatinine	0.21269
Concentration of serum CRP	**–0.79789**
Contribution rate	**19.18%**

RER is a marker of T cells chronically exposed to asbestos, these may be unknown biological mechanisms between immunocompetent cells with chronic exposure to asbestos and peripheral platelet counts via PDGF.

IL-10 and TGF-ß concentrations in MM patients and the experimental model

As shown in the upper panels of Figures 1-B and 1-C, plasma concentrations of IL-10 and transforming growth factor (TGF)-ß were significantly higher in MM patients than in healthy volunteers. TGF-ß is known as one of the mesothelioma cell-producing cytokines (Gerwin et al. 1987; Maeda et al. 1994). However, the above-mentioned MT-2Rst cells, representing the outcome of the experimental low-dose and long-term asbestos-exposure T-cell model, showed significantly higher secretion of TGF-ß than MT-2Org cells (lower panel of Fig. 1-C). As we mentioned previously, the IL-10 concentration in culture supernatants of MT-2Rst was higher than that of MT-2Org (lower panel of Fig. 1-B). Thus, the source of the elevated TGF-ß and IL-10 concentrations in MM patients is not only tumor cells, but also immunocompetent cells.

It is interesting to note that IL-10 and TGF-ß are the important soluble factors necessary for the function of CD4+25+FoxP3+ regulatory T cells (Treg), even though cell-cell contact is the main route for the manifestation of Treg function (Wahl et al. 2004; Romagnasi, 2006). If circulating Treg and tumor-infiltrated Treg have enhanced function as a result of these elevated concentrations of IL-10 and TGF-ß, further aggressive progression of asbestos-induced cancer cells may have occurred. It may be important to analyze the Treg function using the experimental model that we have developed.

T-cell receptor (TcR) Vß expression

We reported previously that asbestos may act on peripheral T cells as a superantigen (Aikoh et al. 1998; Ueki, 2001). The effects of a superantigen such as staphylococcal enterotoxin B (SEB) may modify TcRVß on peripheral T cells to enhance multiple, but not clonal, TcRVß expression (Schubert, 2001; Li et al. 1999). As shown in Figure 2, various TcRVßs were over-expressed in MM and asbestosis patients. TcRVßs from most patients showed a higher expression, exceeding the average plus 2SD (standard deviation) limit. In addition, several TcRVßs such as Vß 1, 4 and 9 among the 24 kinds of TcRVß were strongly over-expressed in many patients. This phenomenon was also observed from the comparison of TcRVß expression in MT-2Org and MT-2Rst cell lines. As a result, MT-2Rst cells over-expressed various TcRVßs. Although TcRVß-overexpressing MT-2Org cells underwent apoptosis due to their first contact with chrysotile, MT-2Rst cells showed no significant changes when they again came in contact with chrysotile (Nishimura et al. 2006). These findings may suggest that the over-expression of various TcRVßs may be the result of contact between cells and chrysotile, an asbestos fiber, during the acquisition of resistance to CB-induced apoptosis caused by a long-term and low-dose exposure to CB (Nishimura et al. 2006).

The multiple over-expression of TcRVß in CD3+ peripheral T cells derived from asbestos-exposed patients may be one of the candidates to detect previous asbestos exposure. Although these

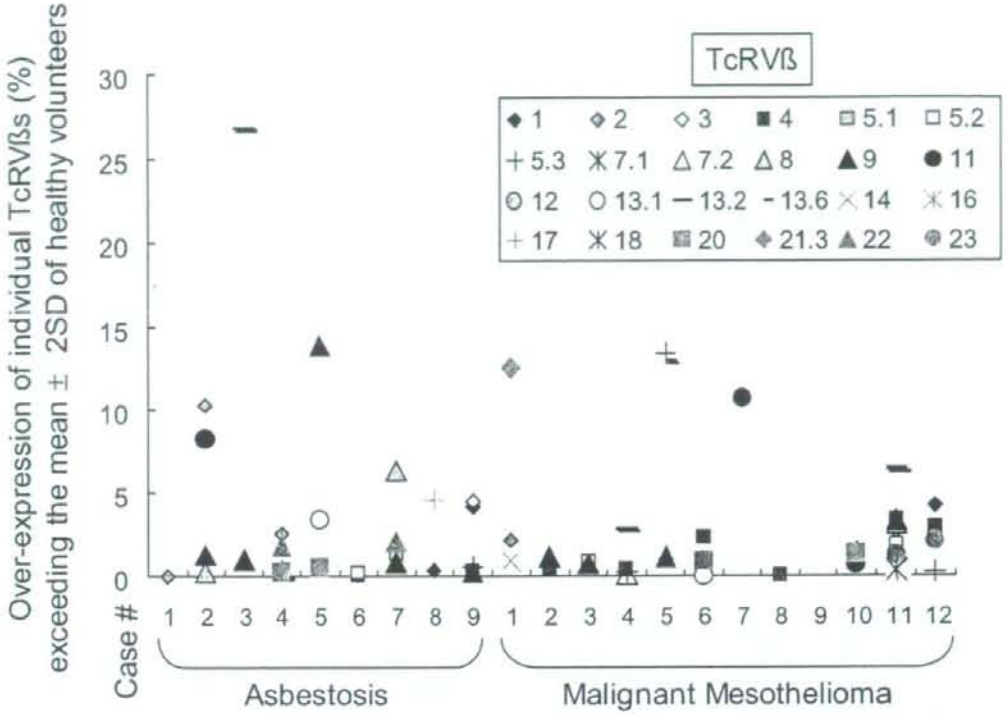

Figure 2. TcRVß expression among patients with asbestosis and malignant mesothelioma. Peripheral blood mononuclear cells (PBMCs) were obtained from 6 HV (mean age ± SD, 38.0 ± 6.4 years old; male(M):female(F),1:5), 9 asbestosis patients without significant clinical signs of complications such as lung cancer or malignant mesothelioma (ASB; 74.4 ± 3.9, all males), and 12 patients with malignant mesothelioma (MM; 58.7 ± 10.1, M:F, 9:3). Specimens were only taken once informed consent had been obtained. The study was approved by the Ethics Committee of Kawasaki Medical School, Okayama Rosai Hospital, Hyogo College of Medicine and Kusaka Hospital. The expression of TcRVß in CD3+ cells derived from HV, ASB and MM subjects was examined with an IOTest Beta Mark TcRVß repertoire analysis kit (Beckman Coulter, Inc., Fullerton, CA) using a FACSCalibur flow cytometer (Becton, Dickinson and Company, Franklin Lakes, NJ) according to the manufacturer's instructions. This kit can analyze TcRVß 1, 2, 3, 4, 5.1, 5.2, 5.3, 7.1, 7.2, 8, 9, 11, 12, 13.1, 13.2, 13.6, 14, 16, 17, 18, 20, 21.3, 22 and 23 from 1 ml of peripheral blood. The 0% expression in this figure is the mean + 2SD for HV. Thus, each symbol indicates the number of over-expressions observed in individual patients for individual TcRVßs.

data were obtained from a limited number of patients, it is worth continuing these analyses using samples from many patients in an effort to explore the biological mechanisms involved in these findings.

Conclusion

A summarized schematic presentation of various aspects of this investigation is shown in Figure 3. This schema only shows the experimental and clinical findings related to asbestos exposure of T cells. We have been investigating the effects of asbestos on the function of natural killer (NK) cells from cellular and molecular viewpoints. TGF-ß is similar to PDGF in that it is also known as a mesothelioma-producing growth factor (Gerwin et al. 1987; Maeda et al. 1994; Langerak et al. 1996; Klominek et al. 1998). Thus, the effects of TGF-ß1 on asbestos-exposed MT-2Rst cells are being investigated and compared with MT-2Rst and MT-2Org cells that have not been exposed to TGF-ß1. These examinations are on-going and will be presented in the near future.

Recent advances in immunomolecular studies have led to detailed analyses of the immunological effects of asbestos. Asbestos affects immunocompetent cells and these effects may be associated with the pathophysiological development of complications in asbestos-exposed patients such as malignant tumors. In addition, immunological analyses may lead to the discovery of new clinical tools for the modification of pathophysiological

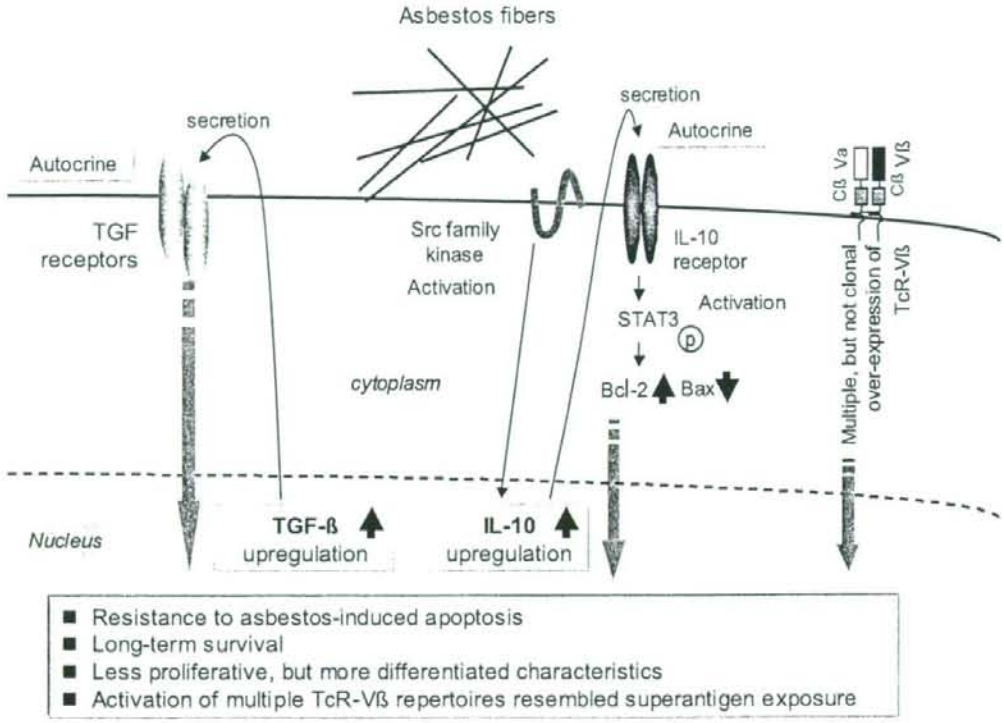

Figure 3. Experimental findings of immunological effects of chrysotile, a form of asbestos, induced by long-term and low-dose exposure using MT-2, an HTLV-1 immortalized human polyclonal T-cell line.

aspects of diseases, such as the regulation of tumor immunity using cell-mediated therapies, various cytokines and molecule-targeting therapies. As the incidence of asbestos-related malignancies increases against the growing concern in Japan since the summer of 2005 for medical and social problems created by such malignancies, efforts should be focused on developing a cure for these diseases in order to eliminate the nationwide anxiety concerning these malignancies.

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Immunological alterations found in mesothelioma patients and supporting experimental evidence

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Abstract It is common knowledge that exposure to asbestos causes asbestos-related diseases, such as asbestosis, lung cancer and malignant mesothelioma, not only in people who have had long-term contact with asbestos in their work environment but also in residents living near factories that handle asbestos. Since the summer of 2005, these revelations turned into a large medical problem and caused and social unrest. We have focused on the immunological effects of both asbestos and silica on the human immune system. In this brief review, we introduce immunological alterations found in patients with malignant mesothelioma and describe the experimental background in which these were found. Analyzing the immunological effects of asbestos may improve our understanding of the biological effects of asbestos.

Keywords Asbestos · Chrysotile · Immunology · Mesothelioma

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Introduction

Asbestos is a generic name for a group of silicate minerals (complexes containing metals, such as iron and magnesium), the most common of which are chrysotile, crocidolite, and amosite. Patients exposed to asbestos develop pulmonary fibrosis, commonly called asbestosis, mesothelial plaque and malignant diseases, such as lung cancer and malignant mesothelioma (MM) [1–3]. Some of these malignancies may be considered to be a result of a decline in tumor immunity owing to the exposure of immunocompetent cells to asbestos.

Silica is known to be one of the most hazardous environmental substances in terms of causing autoimmunity dysfunction [4–6]. Silicosis patients often develop immunological complications, such as rheumatic arthritis (known as Caplan syndrome; [7–9], systemic sclerosis (SSc) and systemic lupus erythematoses (SLE). The effects of silica on autoimmunity have also been assumed as patients who receive plastic surgery with implants containing silicone ($[SiO_2–O–]_n$) also frequently develop complications related to autoimmune disorders [10–12]. Taken together, these findings clearly indicate that crystalline silica causes dysregulation and/or disturbance of the human immune system in general, and of autoimmunity in particular.

Asbestos may affect human immunocompetent cells, and these alterations may, in turn, influence the occurrence and progression of asbestos-related malignant diseases, such as MM. For this reason, this article focuses on the immunological effects of asbestos. Chrysotile has been the most commonly used type of asbestos in our experiments. Magnesium, the main compartment of chrysotile as a silicate, usually dissociates from the chrysotile core, SiO_2, in the human body

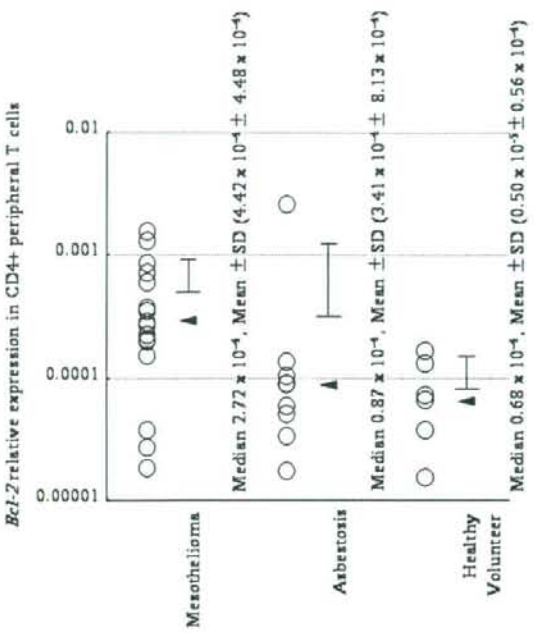

Fig. 1 Comparison of *bcl-2* relative expression ratio (RER) in peripheral blood CD4+ T cells among healthy volunteers, asbestosis patients and malignant mesothelioma (MM) patients. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood of healthy donors and from patients with asbestosis and MM using a Ficoll–Hypaque density gradient (Separate-L; Muto Pure Chemicals, Tokyo, Japan). For the isolation of CD4+ T cells, PBMCs were further separated using magnetic cell separation (MACS) CD4 MicroBeads (Miltenyi Biotech, Bergisch Gladbach, Germany) according to the manufacturer's instructions. The enriched cells were >90% pure as determined by flow cytometry. Specimens were taken from healthy volunteers and patients from whom informed consent had been obtained. The Institutional Ethics Committee of Kawasaki Medical School, Hyogo College of Medicine, and Okayama Rosai Hospital approved the project. A fluorescence thermocycler (Mx3000P QPCR System; Stratagene, La Jolla, CA) was used for real-time reverse transcriptase (RT)-PCR experiments following the instructions of the manufacturer. With this technique, the fluorescence-labeled amplification product is measured continuously. Total RNA obtained from CD4+ T cells isolated from peripheral CD4+ T cells was extracted using an RNA Bee kit (Tel-Test, Friendswood, TX), and 5 μg of RNA was reverse-transcribed with standard methods using a RevertAid H Minus First Strand cDNA Synthesis lit (Fermentas, Ontario, Canada). An amount of cDNA equivalent to 50 ng of RNA served as the template for PCR in a volume of 20 μl (each primer and SYBER Premix Ex Taq; TaKaRa, Japan). The primers for *bcl-2* and *gapdh* were added to the same reaction tube at the optimal concentration for each primer set, and PCR was performed. The primers were as follows: Bcl-2 [5′-TGATGTGAG TCTGGGCTGAG-3′ (forward: Fw) and 5′-GAACGCTTTGTCCA GAGGAG-3′ (reverse, Rv)]; Bax [5′-AGTAACATGGAGCTGCA GAGG-3′ (Fw) and 5′-ATGGTTCTGATCAGTTCCGG-3′ (Rv)]; GAPDH [5′-GAGTCAACGGATTTGGTCGT-3′ (Fw) and 5′-TTGA TTTTGGAGGGATCTCG-3′ (Rv)]. The relative expression of various target genes, such as *bcl-2*, was calculated when real-time RT-PCR was performed. The relative level of the target gene is expressed as $\frac{1}{2}[B - A]$, with *gapdh* expression being 1.0, and A = number of PCR cycles required to reach a certain intensity of fluorescence for the *gapdh* product, and B = number of PCR cycles required to reach the same fluorescent intensity for the target gene product (*bcl-2*) derived from the same sample. PCR products were confirmed to be successfully amplified by standard agarose gel electrophoresis and staining with ethidium bromide. Comparisons of the results for relative gene expression and proliferation assayed by real-time RT-PCR were analyzed using Fisher's parametric least significant difference (PLSD) test

after inhalation. Chrysotile is known to induce malignant transformation, but its carcinogenic capacity is lower than that of iron-containing asbestos, such as crocidolite and amosite [13–15].

We report here the immunological alterations of MM patients in an experimental background. These changes may have resulted from the immunological effects of asbestos on human immunocompetent cells and, as such, may provide valuable information that can be used in preventing the immunological changes seen in the occurrence and progression of asbestos-induced malignant diseases.

bcl-2 expression of peripheral CD4+ T cells in MM patients

Figure 1 shows that peripheral CD4+ T cells from MM patients express significantly more *bcl-2* than healthy volunteers and asbestosis patients [16]. This may suggest that the overexpression of *bcl-2* in peripheral CD4+ T cells is one of the markers of the occurrence of MM in asbestos-exposed patients, although further study is necessary to determine whether many other cancer-bearing patients show the same change. The experimental background leading up to this finding is as follows.

We first exposed peripheral fresh T cells or T cell-derived cell lines to high doses of chrysotile for a short time and found that a human T-cell leukemia virus type-1 (HTLV-1)-immortalized human polyclonal T cell line, MT-2, underwent apoptosis. This apoptosis progressed with reactive oxygen species (ROS) production via activation of the mitochondrial apoptotic pathway with the phosphorylation of p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK) signaling molecules. In addition, short-term, high-level exposure to chrysotile resulted in a shift of the Bax-dominant Bax/Bcl-2 balance followed by the release of cytochrome-c from mitochondria into the cytosol and the activation of caspases 9 and 3 [17]. These observations led us to believe that an in vitro experimental model of chronic exposure was necessary both to analyze the immunobiological effects of