

Table 1 Laboratory data on admission

Hematology					
WBC	18,600/ μ l	Na	145 mEq/l	HIV Ab	negative
Baso	0.0%	K	3.5 mEq/l	Influenza B Ab (CF)	\times 512
Eosino	0.0%	Cl	103 mEq/l	Aspergillus Ag (EIA)	0.3 ng/ml
Neutro	95.5%	BUN	30 mg/dl		
Lymph	1.0%	Cr	0.7 mg/dl	Blood gas analysis	(O ₂ 3L)
Mono	1.0%	FBS	184 mg/dl	pH	7.5
RBC	2.55×10^6 / μ l	HbA1c	5.7%	PaO ₂	88 torr
Hb	9.0 g/dl			PaCO ₂	41 torr
Ht	27.8%	Serology		HCO ₃	31 mml/l
Plt	27.2×10^4 / μ l	CRP	8.9 mg/dl	SaO ₂	96.5%
		ESR (1h)	47 mm/1h		
Biochemistry		IgG	1,538 mg/dl	Sputum culture	
TP	6.1 g/dl	IgA	144 mg/dl	Bacteria	(-)
Alb	2.1 g/dl	IgM	82 mg/dl	Acid fast bacteria	(-)
GOT	111 IU/l	BNP	366 pg/ml	Fungi	(-)
GPT	185 IU/l	KL-6	1,012 U/ml		
LDH	286 IU/l	s-IL2R	2,080 U/ml	Urinary	
γ -GTP	78 IU/l	β -D glucan	12.3 pg/ml	Legionella Ag	negative
				S. pneumoniae Ag	negative

が亢進していた。また末梢血リンパ球数は、186/ μ lと著明に低下していた。一方、生化学検査では、低アルブミン血症、肝機能障害、BUNの上昇を認めた。血液ガス所見では、酸素3L鼻カニューラ下でPaO₂88Torr、PaCO₂41Torrであった。

喀痰培養では、一般細菌、抗酸菌および真菌共にすべて陰性で、尿中肺炎球菌抗原ならびに尿中レジオネラ抗原はともに陰性であった。HIV抗体は陰性で、血清アスペルギルス抗原は0.3、 β -Dグルカン12.3pg/mlと共に正常範囲内であった。また前医で検査された血清B型インフルエンザウイルス抗体(CF法)が512倍と上昇しており、最近の同ウイルス感染が疑われた。

画像検査所見：2月22日の他院初診時の胸部X線写真では、右中肺野に浸潤陰影、左下肺野に結節陰影、右上肺野ならびに両側下肺野に網状陰影を認めた(Fig. 1)。同時期の胸部CT写真では、右中葉および左舌区にair bronchogramを伴うconsolidationを認め、右上葉ならびに両側下葉を中心にスリガラス状陰影、両側肺底部胸膜下に蜂巣肺を認めた(Fig. 2)。

入院後経過：2月28日に前医で施行したTBLB標本からアスペルギルス菌糸が多数認められたことよりIPAと診断し、3月5日より1日あたりアンホテリシンB (AMPH-B) 50mg、ミカファンギン(MCFG) 150mg、イトラコナゾール(ITCZ) 400mgを併用投与した。また画像上、IPFの急性増悪の合併も疑い、ステロイドパルス療法後もPSL 20mgを継続投与した。しかしながら臨床症状、画像所見ならびに血液ガス所見の改善は認められず、呼吸不全の進行に対し第16病日に気管内挿管

し、人工呼吸器管理を開始した。その後、腎機能障害が出現したため、AMPH-Bを中止し、MCFGを300mg/日に増量したが、第21病日に呼吸不全で死亡した(Fig. 3)。

病理所見：

1) TBLB検体：2月28日に施行された右中葉の肺炎像に対するTBLB検体では、Y字状の分岐を示し、隔壁を有するアスペルギルス菌糸を多数認めた(Fig. 4)。

2) 剖検肺：肉眼的には右中葉に4 \times 3cm大、左舌区に2 \times 2cm大の黄白色調の境界明瞭な結節性病変が認められた。内部は一部壊死により空洞化しており、膿瘍と考えられた。また両側肺底部には、蜂巣肺を認め、その周囲の肺実質は気腔の拡張と粘稠な滲出物で充満されていた(Fig. 5)。組織学的には、膿瘍病変の内部には、多数のアスペルギルス菌糸が、放射状かつ緻密に認められた。右中葉では、肺動脈への血管内侵襲、右下葉では肺胞内に菌糸が集簇する所見を認めた(Fig. 6)。また肺実質はDADの組織像を示し、肺胞腔内には器質化を伴う滲出物と、一部硝子膜の形成が混在して認められた。両側肺底部には、蜂巣肺が形成されていた(Fig. 7)。さらに咽頭から気管および両側主気管支粘膜は高度のびらんを呈し、一部に細菌塊の付着が認められた。一方、末梢の細気管支領域においては、アスペルギルスによる膿瘍の領域を除いて、粘膜病変は認められなかった。

以上より、本症例は、肺の基礎疾患としてIPF/UIPを有し、インフルエンザ感染を契機に侵襲性肺アスペルギルス症およびDADを併発したものと考えられた。

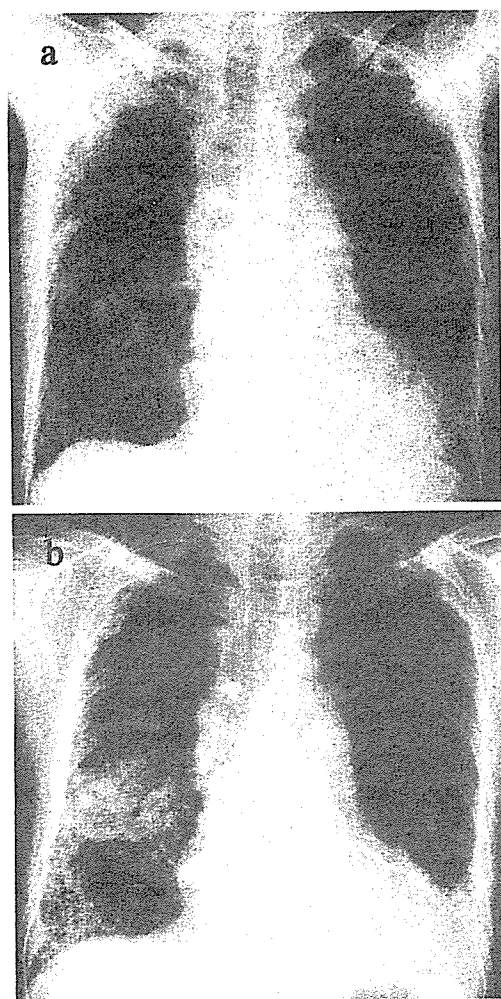


Fig. 1 Chest radiographs. a) Taken on February 22, 2005, showing consolidation in the right middle lung field and reticular opacities in the bilateral lower lung fields. b) Taken on admission March 4, showing progressive infiltrative and reticular shadows in the right lung, and consolidation in the left middle lung field.

考 察

1990年にフルコナゾールが臨床導入されて以来、剖検例で認められるカンジダ症は減少傾向にあるが、その一方で重症型肺アスペルギルス症、とくにIPAの増加が指摘されている¹⁾。IPAは、深在性真菌症の中でも最も重篤な疾患である。空気中に浮遊したアスペルギルス属の分生子を吸入することで肺に感染巣が形成される³⁾が、本疾患の発症には、肺胞マクロファージ、好中球、気道粘液線毛クリアランスの障害ならびに菌自体が産生する種々な物質が関与するといわれている⁴⁾。そのため危険因子としては、好中球減少が遷延した患者⁵⁾、骨髄

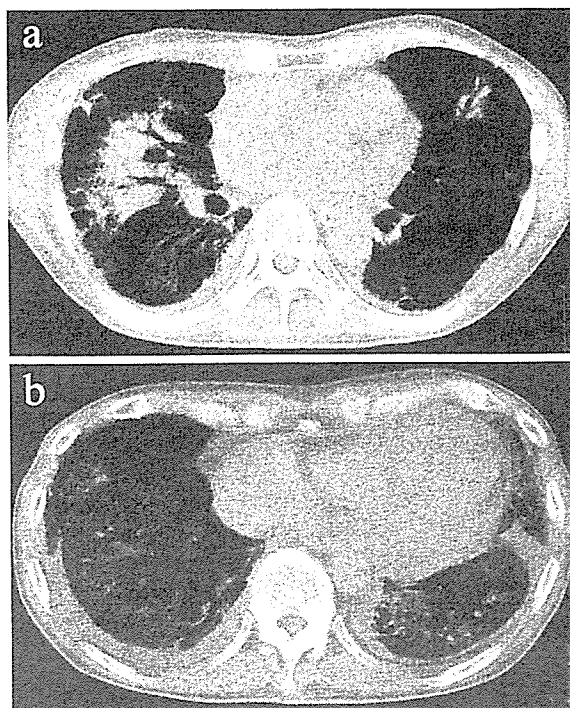


Fig. 2 Chest computed tomography scans. a) On admission, showing consolidation in the right middle lobe and in the lingula. b) Ground-glass opacity associated with honeycomb lesions in the bilateral lower lobes. Slight pleural effusion was present.

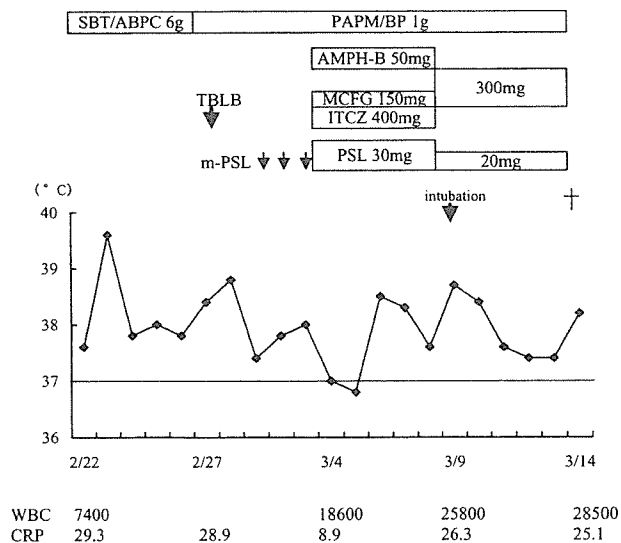


Fig. 3 Clinical course of the presented case

移植⁶⁾、ステロイドの大量および長期投与⁷⁾、AIDSや慢性肉芽腫症のような後天性あるいは先天性免疫不全⁸⁾などが挙げられる。しかし、稀ではあるが明らかな免疫不

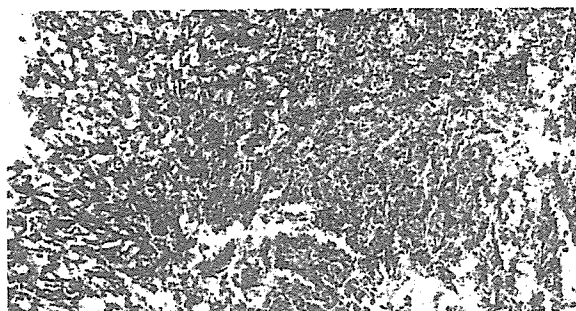


Fig. 4 Transbronchial lung biopsied specimen, showing numerous hyphae with Y-shaped branching in the necrotic area suggestive of aspergillus infection. (Hematoxylin-eosin stain, $\times 200$).

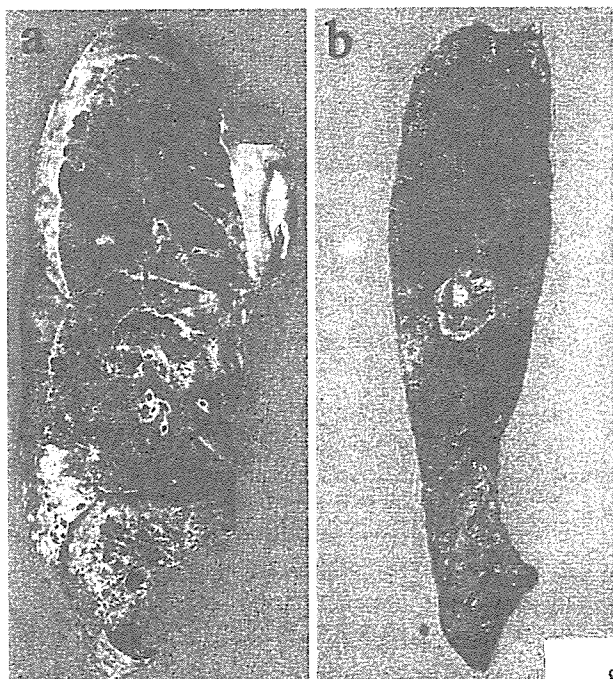


Fig. 5 Macroscopic appearance of the right middle lobe (a) and the lingula of the left upper lobe (b), showing segmental necrosis with cavitation (arrows).

全を伴わない宿主にも発症することが報告されている²⁾。その中で、本症例のようなインフルエンザウイルス感染後に IPA を合併したとするものが過去に 11 例報告されている^{9)~13)} (Table 2)。これらの報告をまとめると、中高年に多く、発症時の平均年齢は 55 歳で、男女比は 7:4、8 例で基礎疾患がなく、基礎疾患を有した 3 例の内訳は糖尿病、アルコール中毒、塵肺各 1 例であった。ステロイド剤の使用は 1 例のみであったが、一般抗菌薬は 10 例に使用されていた。発症時の末梢血リンパ球数の平均は $962/\mu\text{l}$ (160~2,499) で、7 例が $1,000/\mu\text{l}$ 未満で



Fig. 6 Microscopic findings of the lung. a) Small pulmonary artery was invaded by hyphae of aspergillus in the right middle lobe (arrow). (Hematoxylin-eosin stain, $\times 20$). b) A necrotic lesion with infiltration of neutrophils and hyphae of aspergillus radially arranged around it seen in the lingula of the left upper lobe (arrow head). (Hematoxylin-eosin stain, $\times 40$). c) Intra-alveolar space was filled with hyphae of aspergillus observed in the right lower lobe (arrow). (Hematoxylin-eosin stain, $\times 100$).

あった。インフルエンザウイルスの血清型は 10 例が A 型、1 例のみが B 型であった。

IPA の診断方法としては、病巣部の生検は 3 例のみで、その他は喀痰、経気管吸引痰、気管支洗浄液、血清学的検査に基づいて診断されていた。剖検は 7 例で行われており、主な病理組織所見として、気管支肺炎・膿瘍が 4 例、梗塞・出血が 2 例、器質化肺炎・空洞が 1 例に認められた。抗真菌薬治療は 11 例中 8 例で行われており、AMPH-B が 7 例と最も多く投与されていた。予後は不良で、9 例 (82%) が呼吸不全で死亡していた。また発症要因として、インフルエンザウイルス感染後のリンパ球減少および肺胞マクロファージの活動低下などによる細胞性免疫の低下¹⁹⁾や、気道線毛系の障害¹⁶⁾が IPA の発

Table 2 Reported cases of invasive pulmonary aspergillosis associated with influenza

No.	Age	Sex	Underlying disease	Steroid	Antibiotics	Type of influenza	Lymphocyte counts	Diagnosis	Histopathological findings of lung	Therapy	Outcome	Reference number
1	51	F	-	-	+	NM	270	Autopsy	Bronchopneumonia, Cavity, Hemorrhage	-	Died	9)
2	59	F	-	-	+	A	950	Sputum, Biopsy, Autopsy	Bronchopneumonia, Pulmonary infarction	-	Died	10)
3	47	F	-	-	-	A	675	Sputum, Autopsy	Bronchopneumonia, Pulmonary infarction	-	Died	10)
4	69	F	-	-	+	A	1,300	Tracheal aspiration	-	AMPH-B, 5FC, Econazole	Survived	11)
5	42	M	DM, HT	-	+	A	850	Bronchial washing, Autopsy	Pneumonia, Abscess	AMPH-B	Died	12)
6	28	F	-	+	+	A	1,520	Sputum, Biopsy, Autopsy	Organizing pneumonia, Abscess	AMPH-B	Died	13)
7	61	M	-	-	+	A	NM	Tracheal aspiration, Autopsy	Necrotizing pneumonia, Abscess	AMPH-B	Died	14)
8	49	M	Alcoholism	-	+	A	2,499	BAL, Biopsy	Abscess	AMPH-B, MCZ, FLCZ	Died	15)
9	83	F	-	-	+	A	160	Serology	-	AMPH-B, ITCZ	Died	16)
10	55	M	Pneumococcosis	-	+	A	434	Sputum, Autopsy	Abscess, Hemorrhage	FLCZ	Died	17)
11	63	F	-	-	+	B	NM	Sputum, Serology	-	AMPH-B, ITCZ	Survived	18)
12	56	M	Esophageal Ca, IPF	-	+	B	399	TBLB, Autopsy	Abscess, Cavity, Diffuse alveolar damage	AMPH-B, MCFG, ITCZ	Died	Present case

NM; not mentioned, DM; diabetes mellitus, HT; hypertension, IPF; idiopathic pulmonary fibrosis, AMPH-B; amphotericin B, 5FC; flucytosine, MCZ; miconazole, FLCZ; fluconazole, ITCZ; itraconazole, MCFG; micafungin, BAL; bronchoalveolar lavage, TBLB; transbronchial lung biopsy

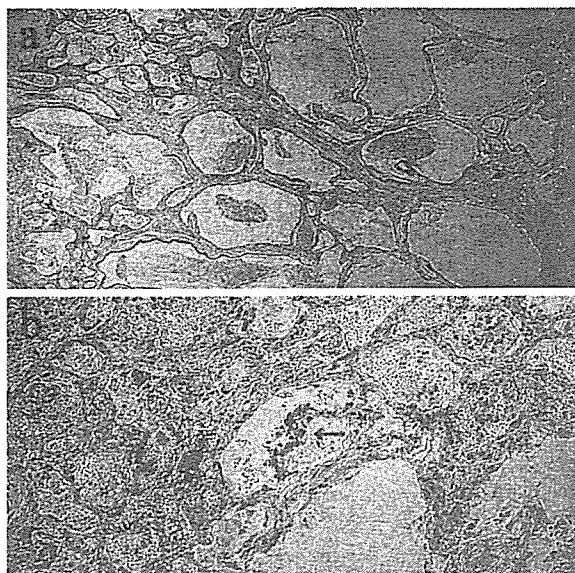


Fig. 7 Microscopic appearance of the lung base and the adjacent area. a) Honeycomb lesions. (Hematoxylin-eosin stain $\times 20$). b) Diffuse alveolar damage and exudation of hyaline membrane (arrow). [Same as a), but $\times 100$].

症に影響を与えたのではないかと推察されていた。

本症例では、血清B型インフルエンザウイルス抗体が512倍と上昇しており、最近の同ウイルス感染が疑われた。一方で、入院3カ月前の末梢血リンパ球数は2,200/ μl と正常範囲であったが、今回発症時のリンパ球数は400/ μl まで低下しており、諸家の報告にあるようにインフルエンザウイルス感染とIPA発症との関連性が示唆された。また福家ら²⁰⁾は、IPF患者のBALF中のSP-AおよびSP-Dが健常者より低値であることを指摘し、このことが宿主側の肺局所免疫能を低下させ、IPFにIPAが合併した誘引になったのではないかと推察している。したがって本症例でも同様に、先行する肺疾患としてIPFが存在し、肺局所の感染防御能を低下させていたのではないかと考えられた。さらに経過中にIPFの急性増悪に対するステロイドパルス療法ならびに継続的にステロイド投与を余儀なくされていたことが、本疾患の発症あるいは、その後の病態をいっそう悪化させた一要因になったと考えられた。一方で、本症例のように基礎疾患に肺線維症を有する症例がIPAを発症したとする報告は、我々が検索した限りでは、2例のみであった²⁰⁾²¹⁾。両症例とも70歳代の男性で、ともにステロイド剤などの免疫力を低下させる薬物の使用は行われていなかった。画像所見は、宿主の状態に修飾されて多様であり、単発もしくは多発する結節影、浸潤影、胸膜側を底辺とする楔状浸潤影が挙げられる²²⁾。さらに発症初期に

認められる halo sign²³⁾や、回復期の空洞形成、すなわち air crescent sign²⁴⁾を呈することも画像上の特徴の1つとされる。Shibuyaら²⁵⁾は、ヒトのIPAの病変を好中球浸潤のほとんど認めない凝固壊死からなる結節性病変と、種々の程度の好中球浸潤と融解壊死からなる気管支肺炎様の病変の2者に大別し、これらが好中球機能の減弱の程度に規定されることを指摘した。本症例では、全経過を通じて好中球は正常あるいは増加しており、好中球反応により融解壊死を主体とした膿瘍を形成し、気管支ドレナージにより空洞を形成したものと考えられた。

本症例では、一般抗菌薬不応性の肺炎に対して、入院1週間後に気管支鏡検査が施行され、比較的早期にIPAの診断に至ったが、最終的には発症から21日後にDADによる呼吸不全で死亡した。一方、抗原検査法としてEIA法を用いたが、本症例では陰性であり、やはり感度の点で問題が残った。本症例のDADの原因としては、IPAあるいはインフルエンザウイルス感染によるARDS、既存のIPFの急性増悪などが鑑別に挙げられたが、いずれかを確定することは困難であった。本症例のように、明らかな免疫不全が認められない患者においても、インフルエンザウイルス感染後に急速進行性に悪化する治療抵抗性の肺炎を認めた場合は、IPAを鑑別疾患の1つに挙げるべきと考えられた。

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Abstract

Fatal invasive pulmonary aspergillosis triggered by influenza B virus infection in an individual with idiopathic pulmonary fibrosis

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A 56-year-old man with idiopathic pulmonary fibrosis developed acute symptoms, including fever and cough. Chest X-ray and CT scan films revealed consolidations and ground glass opacities in the bilateral lungs suggestive of massive pneumonia and acute respiratory distress syndrome (ARDS). Microscopic examination by a transbronchial lung biopsy from the right middle lobe demonstrated numerous hyphae of aspergillus. Despite treatment with anti-fungal agents, respiratory failure rapidly progressed, and the patient died on the 21st hospital day. Post-mortem examination of the lung showed multiple abscesses with hyphae of aspergillus invading into small pulmonary artery and alveoli, which were the characteristic findings of invasive pulmonary aspergillosis (IPA). In addition, diffuse alveolar damage was also present, associated with the typical features of UIP such as honeycombing in the bilateral lungs. The serum anti-influenza B virus antibody was high at 512-fold. Therefore, it was speculated that influenza B virus infection triggered superimposed aspergillus infection leading to fatal IPA associated with ARDS.

びまん性汎細気管支炎, 線毛不動症候群の病理と病態

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はじめに

びまん性汎細気管支炎 diffuse panbronchiolitis (DPB) および線毛不動症候群 immotile-cilia syndrome は、共に副鼻腔気管支症候群 sino-bronchial syndrome の臨床像をとることが注目され、気道系の防御機構の欠損・低下が病因と考えられている。

DPB は呼吸細気管支を病巣の主座として両側肺にびまん性に分布し、徐々に呼吸不全に進行する予後不良の疾患であるが、近年、エリスロマイシン少量長期投与により DPB は著減しており、画像診断の向上と共に病理像をみる機会は少なくなっている。しかし、治療抵抗性の症例などに遭遇する機会もあり、その病理ならびに病態を理解することは重要である。

線毛不動症候群は、線毛の超微構造異常に基づく線毛の機能不全症であり、近年では primary ciliary dyskinesia (PCD) と称されている。気管支拡張症、内臓逆位、慢性副鼻腔炎を三徴とする Kartagener 症候群は PCD の約 50% にみられ、線毛不動症候群のサブグループととらえられており、Kartagener 症候群における内臓逆位と線毛との関連性について知見が集積されてきている。

本稿では、DPB ならびに線毛不動症候群 (Kartagener 症候群を含む) の病理および病態、さらに鑑別疾患として、びまん性嚙下性細気管支炎、濾胞性細気管支炎、HTLV-I 関連細気管支・肺胞異常症 (HABA) について概説する。

I. びまん性汎細気管支炎 (DPB)

1. 概念と病因

本間、山中らにより症例の集積と概念の確立がなされた疾患であり¹⁾、ほとんどが日本を含む東アジアの諸民族に認められ、欧米人では極めて稀である。診断の手引きは厚生科学研究びまん性肺疾患研究班により 2002 年に改定されている²⁾。DPB の病因は未だ不明であるが、家族発生頻度の高いことから遺伝性の関与が示唆されており、我が国の DPB 患者ではクラス I 抗原の HLA-B54 が高率に認められる。

2. 臨床的事項

発症は各年齢層に広く分布し、男女の差はほとんどなく、発症のピークは 40~50 歳代である。多くの例では小児期からの慢性副鼻腔炎の合併または既往があり、咳、喀痰、労作時息切れなどの呼吸器症状が進行する。また、インフルエンザ菌や肺炎球菌などの感染に伴って組織破壊が進行し、細菌感染→組織破壊→細菌感染という悪循環が形成され、二次的に中枢側の気管支拡張を起こし、病状がさらに進行すると思われる。初期の胸部単純 X 線写真では両側肺野にびまん性散在性粒状陰影および肺の過膨張を呈し、CT では小葉中心性の粒状陰、線状影を認める (図 1)。

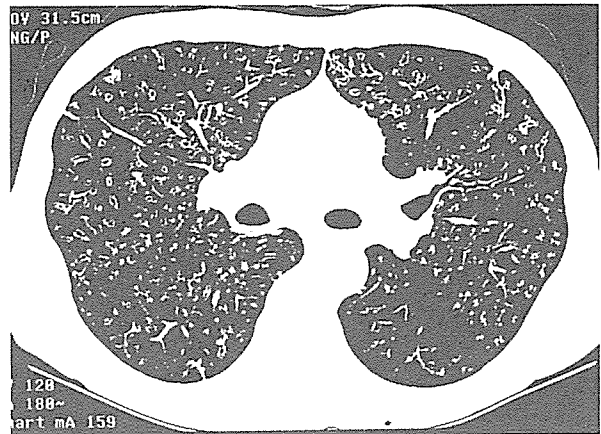
3. 病理学的所見

典型例では小葉もしくは細葉中心性に 2~8 mm 大の淡黄色から灰白色を呈する小結節がみられ (図 2)、進行した例では肺の過膨張を伴う。組織学的には呼吸細気管支領域に一致し (図 3)、リンパ球を主体とする炎症細胞の集簇があり、壁の肥厚、内腔の狭窄を生じ (図 4)、周囲の間質には泡沫細胞集簇 (xanthoma) を認める (図 5)。炎症細胞浸潤は周囲の肺胞に及ぶ場合がある。癒痕化すると灰白色調を呈するようになり、周囲の肺胞領域は過膨張を呈する (図 6)。病変部から中枢側では、呼吸細気管支レベルの狭窄もしくは閉塞

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a



b

図1 DPBの画像所見 a:胸部単純X線像、両側下肺野を主体とした粒状影、線状影、網状影を認め、過膨張を呈している。b:胸部CT像、両側肺野に小葉中心性のびまん性粒状影および線状影を認める。

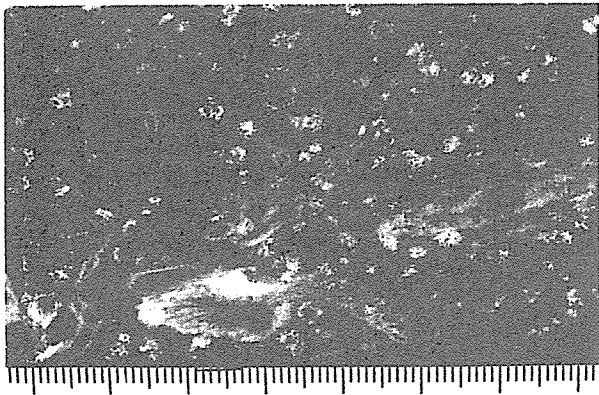


図2 DPBの肉眼像 小葉ないし細葉の中心部に一致して約5mm大までの淡黄色調の結節が分布している。



図3 DPBのルーベ像 病変の分布は呼吸細気管支領域に一致している。

によって細気管支・気管支の拡張を伴う(図7)。しかし、多くの剖検例では、長い経過中に感染を繰り返しており、気管支・細気管支肺炎、気管支粘膜の肥厚、気管支腺肥大、杯細胞の増加といった二次的な変化が加わり、複雑な所見を呈していることが多い。

なお、DPBの定型的所見として斎木による分類³⁾があり(表1)、特に組織学的所見はI~IV型に分類され、多彩な病変を呈するDPBの組織像を考えるうえで有

用性が高い。

II. 線毛不動症候群

1. 概念と病因

Eliassonらによって報告された線毛の先天的系統的形態機能異常で⁴⁾、線毛の機能不全症を呈する疾患であるが、症例の集積により、線毛運動の完全な欠落で

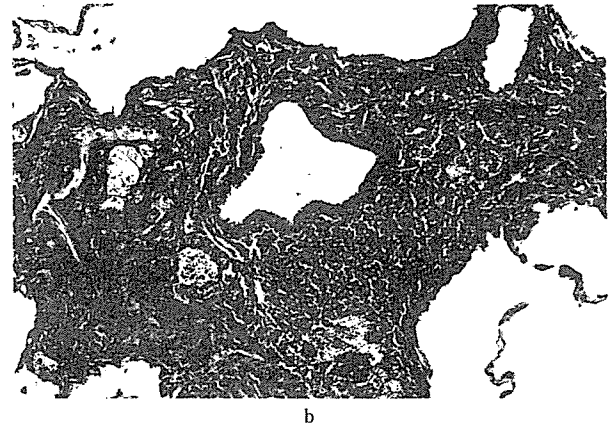
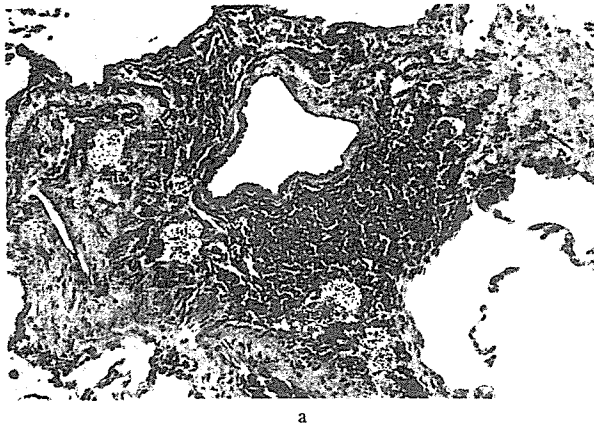


図4 DPBの組織像 呼吸細気管支壁にリンパ球の集簇を認め、壁の肥厚、内腔の狭窄を伴っている。b: elastica van Gieson (EvG) 染色。

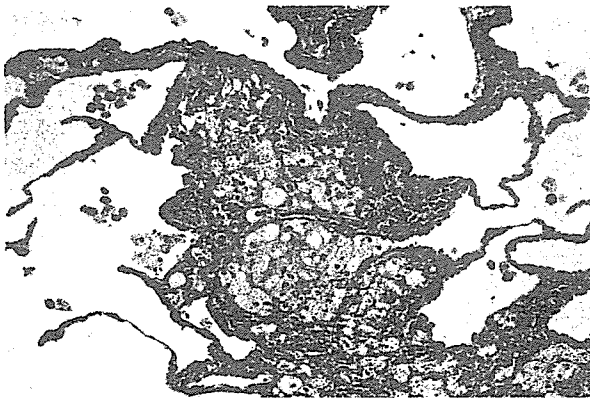


図5 DPBの組織像 呼吸細気管支周囲の間質および肺胞壁内に泡沫細胞集簇を認める。



図6 DPB進行例の組織像 瘢痕化した呼吸細気管支を認め、周囲の肺胞領域には過膨張が加わっている (EvG 染色)。

はなく、微弱な線毛運動や協調性のない線毛運動による気道クリアランスの低下が示されている。

正常線毛正切横断面の模式図を図8に示す。辺縁部に9対、中央には2本の微小管が存在し、9+2の構造を呈する。辺縁の微小管にはdynein arms (outer と inner) と称されるかぎ形の構造が2本ずつ付着している。このdynein armsにはATPaseが存在しており、線毛運動のエネルギー供給源として重要である。中央と辺縁の微小管はradial spoke、辺縁の微小管同士はnexin linkで連結されている。これら線毛の超微構造の先天性欠損や機能不全が本症の病因とされ、さまざまなタイプの欠損が報告されているが、dynein armsの完全欠損ないし不完全欠損が最も多いタイプである。図9にouter dynein armsおよびinner dynein armsの欠損を認めた自験例の電顕像を示す。



図7 DPB進行例のルーベ像 呼吸細気管支レベルの狭窄もしくは閉塞に伴い、中枢側の細気管支・気管支の拡張を呈する。

表1 DPBの定型的所見

A. 肉眼的所見	<ol style="list-style-type: none"> 1. 病巣は両側肺に、びまん性に分布するが、下葉に多い傾向がある(画像でも確認できる) 2. 病巣は呼吸細気管支・終末細気管支に一致して分布する(病変は慢性呼吸細気管支炎, 慢性呼吸細気管支周囲炎である) 3. 病巣は2~8 mm大までの小結節性で, 黄色から灰白色を呈する(剖検開胸時, 肺は含気に富み, 過膨張を示し, 肺の退縮は悪く, 経気道的にホルマリン注入の困難なことが多い)
B. 組織学的所見	<ol style="list-style-type: none"> 1. 呼吸細気管支内のリンパ球浸潤, リンパ濾胞形成等による壁の肥厚, 内腔の狭窄を認める(I型) 2. 呼吸細気管支壁内の肉芽形成, ポリプ状の肉芽組織による内腔の狭窄, 閉塞(II型) 3. 呼吸細気管支壁の肉芽組織による広範な狭窄と閉塞(III型) 4. 呼吸細気管支の癒痕形成による広範な狭窄および閉塞(IV型)
C. 呼吸細気管支病変に続発する病変	<ol style="list-style-type: none"> 1. 病巣より末梢の気腔の過膨張(細気管支肺炎の合併があり, その融合もある) 2. 病巣, その周囲の間質, 肺泡腔内の泡沫細胞の集簇(xanthoma)(肉眼的に淡黄色の小結節にみえたものに相当する) 3. 細気管支肺炎後の癒痕形成に伴う気腫性病変があることもある 4. 終末細気管支より高位の細気管支, 小気管支へと慢性炎症が波及し, 拡張を生ずる 5. 終末像として気管支の慢性炎症に伴う二次性の気管支拡張が広範に起こる

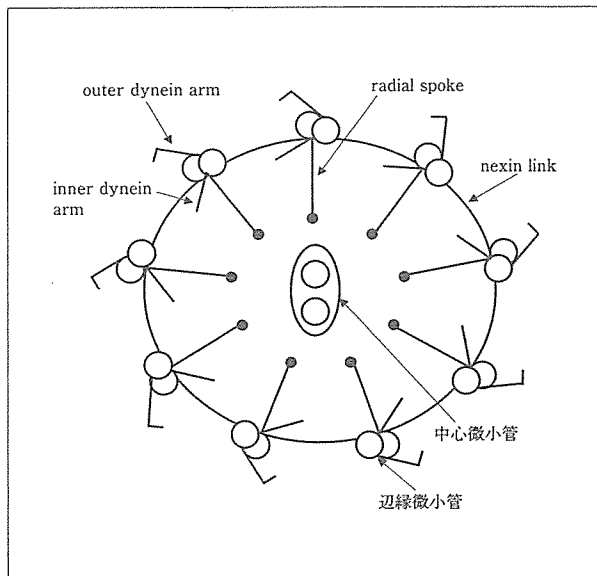


図8 正常線毛正切横断面の模式図

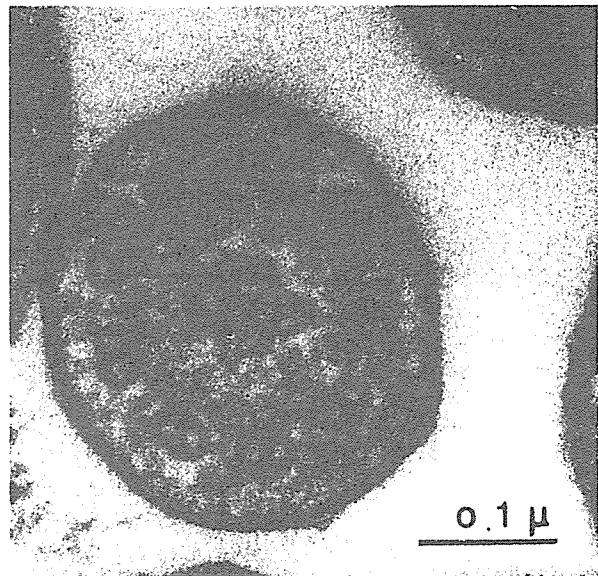


図9 Kartagener症候群の線毛横断面の電顕像 outer dynein arms およびinner dynein armsの欠損がみられる。

遺伝子に関しては, 5つの dynein heavy chain 遺伝子および2つの dynein intermediate chain 遺伝子 (*DNAI1*, *DNAI2*) の研究で, outer dynein arm 欠損と *DNAI1* 異常との関連が指摘され, この遺伝子は染色体9q13-p21に存在することが明らかになっており⁵⁾, 染色体5p15-p14に存在する *DNAH5*, 染色体7p21に存在する *DNAH11* の異常とPCDとの関連についても検討されている^{6,7)}。また, 微小管のフィラメント形成に関与する tektins 蛋白が同定され, その異

常による inner dynein arm の機能不全も報告されている⁸⁾。

Kartagener 症候群における内臓逆位は個体の左右軸決定の異常によるとされ, その左右軸決定のメカニズムと線毛との関連が提唱されている⁹⁾。左右異常変異マウスの原因遺伝子として left-right dynein (*lrd*), さらにモーター蛋白であるキネシン関連蛋白遺伝子のノックアウトマウスにおける左右軸異常が報告され, 特にキネシン *Kif3B* 遺伝子あるいは *Kif3A* 遺伝子の

ノックアウトマウスでは、腹側結節細胞上の単線毛がみられず、心臓の位置異常が認められている^{10,11)}。近年、左右異常を示す変異マウスのもう一つの原因遺伝子として、*inv*の変異体が明らかとなり、クローニングされている¹²⁾。Brodyらは、フォークヘッド遺伝子の*Hh4*ノックアウトマウスで内臓左右異常、気道や鼻腔などの上皮の線毛欠損、精子の鞭毛欠損を確認している¹³⁾。しかし、線毛は既に決定された非対称を維持もしくは拡大するという可能性や、KIF3Bキネシン蛋白における未知の細胞質機能が左右軸決定に関与している可能性もあり、線毛の役割については未だ明らかでない点が多い。なお、ヒトの左右軸異常の原因遺伝子として、心臓大血管位置異常における*ACVR2B*遺伝子、両側左肺を呈する患者での*LEFTYA*遺伝子の突然変異が報告されているが^{14,15)}、Kartagener症候群の原因遺伝子は未だ明らかにされていない。

2. 臨床的事項

常染色体劣性遺伝で、頻度は2万～6万人当たり1人とされている。線毛系器官の異常により、慢性副鼻腔炎、気管支拡張症、上下気道感染症、慢性中耳炎、色素性網膜炎、男性不妊症など多彩な症状が認められるが、呼吸器症状で発見される頻度が高い。自験例のKartagener症候群の胸部画像を図10に示す。単純X線写真ではびまん性の小粒状影、過膨張がみられ、CTではDPBに類似した小葉中心性の小粒状影を認める。マクロライド系抗生物質の少量長期投与の有用性については見解が分かっている。

3. 病理学的所見

症例数が少なく、我々も気管支立体構造再構築の機会に恵まれていないために詳細な検討が不十分であるが、自験例では、呼吸細気管支から膜性細気管支にかけてリンパ球の集簇を伴う炎症細胞浸潤がみられ、呼吸細気管支レベルではDPBと同様、気管支周囲の間質に泡沫細胞集簇を認めた(図11)。膜性細気管支の内腔は拡張し(図12)、少数の好中球および粘液が散見され、二次的な炎症が加わっていた。末梢気管には過膨張所見を認めた。確定診断には、正しく横断されたciliaの電子顕微鏡での観察が重要であるが、二次的な傷害によってもciliaの形態異常が起こるので、可能な限り多くのcilia構造の検索が望ましい。

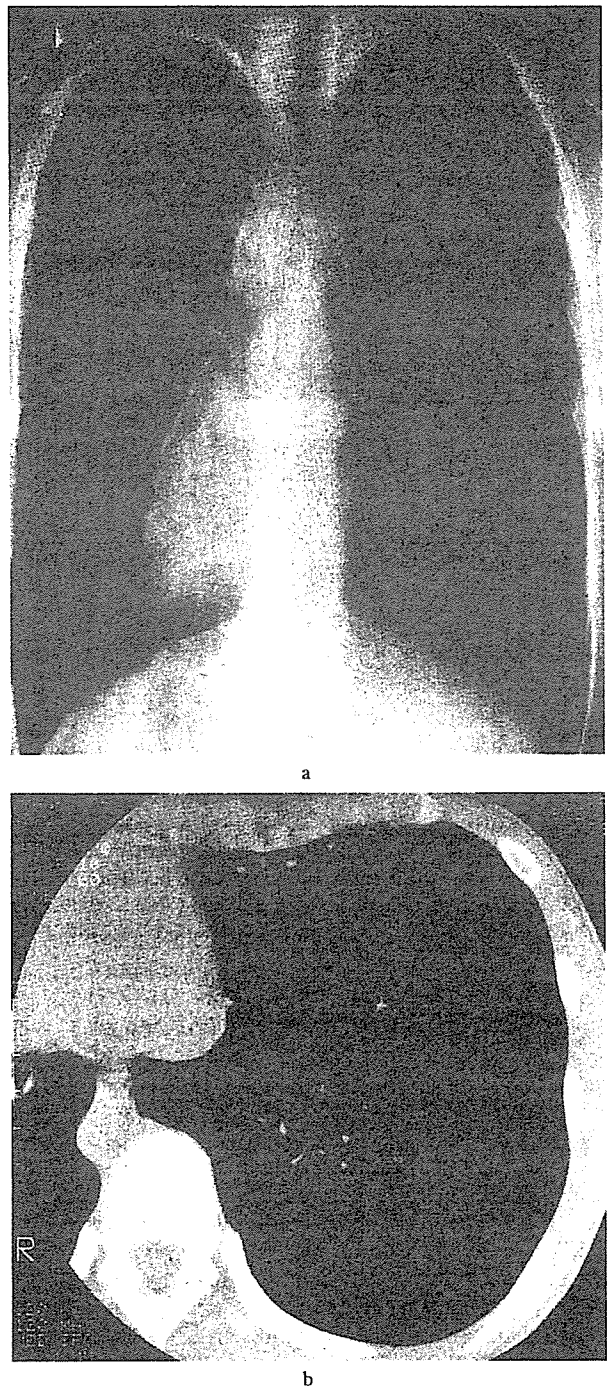
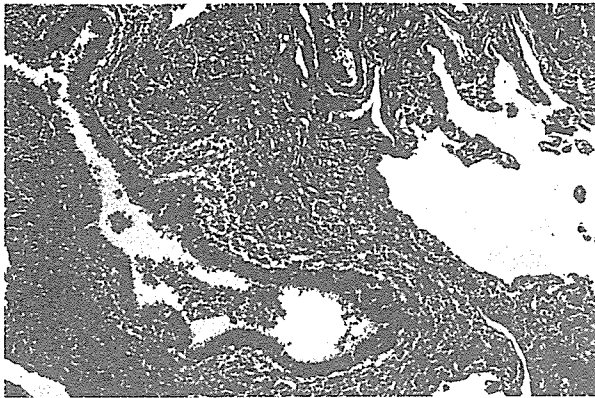


図10 Kartagener症候群の画像所見 a: 胸部単純X線像、右胸心を認め、両側肺野では粒状影、網状影を認める。b: 胸部CT像、肺野には小葉中心性のびまん性小粒状影、線状影を認める。

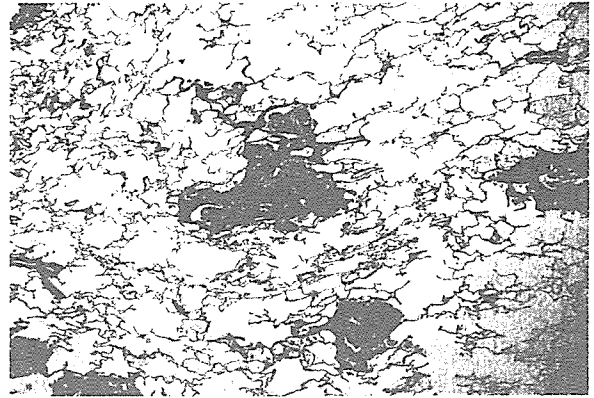


a

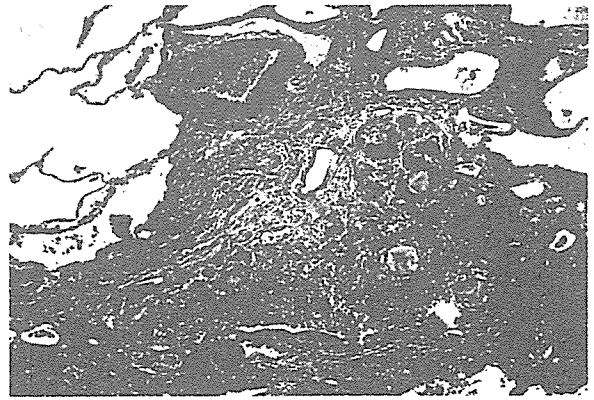


b

図11 線毛不動症候群の組織像 a: 膜性細気管支周囲にはリンパ球を主体とする小円形炎症細胞浸潤を認め、壁の肥厚と内腔の狭窄を認める。b: 細気管支周囲の間質および肺胞壁内の泡沫細胞集簇を認め、DPBと類似している。



a



b

図13 びまん性嗜下性細気管支炎の組織像 a: DPBに類似した小葉中心性の分布を呈する。b: 細気管支から呼吸細気管支腔内に異物型多核巨細胞を散見する。



図12 線毛不動症候群のルーベ像 膜性細気管支の拡張を認める。DPBの中核側の変化と鑑別が難しい。

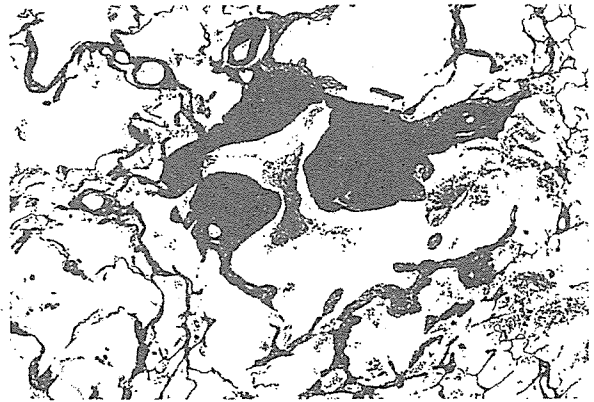


図14 濾胞性細気管支炎の組織像 細気管支壁には胚中心を伴うリンパ濾胞の形成が目立ち、内腔の狭窄を伴っている。

Ⅲ. 鑑別疾患

1. びまん性嚥下性細気管支炎 diffuse aspiration bronchiolitis

高齢者に多く、繰り返す誤嚥の既往が重要である。呼吸困難を呈し、画像上やや大型の粒状影が小葉中心性にみられる。肉眼的にDPBに類似した病変分布を呈するが、組織学的には、呼吸細気管支および反回枝領域にほぼ一致して、異物や異物型巨細胞を確認することで診断が可能である(図13)。

2. 濾胞性細気管支炎 follicular bronchiolitis

細気管支壁に胚中心を有する大きなリンパ濾胞の過形成があり、画像上、DPBに類似した小葉もしくは細葉中心性の粒状影を呈することがある。時に壁の構築が失われ、細気管支内腔の狭窄を伴う(図14)。関節リウマチやSjögren症候群に伴うものが知られているが、さまざまな疾患で認められ、原因が明らかでないものも存在する。

3. HTLV-I 関連細気管支・肺胞異常症(HABA)

HABA (HTLV-I associated bronchiolo-alveolar disorder) 成人T細胞性白血病/リンパ腫患者およびそのキャリアにDPB同様の小葉中心性の細気管支病変が発生することが知られている。DPB 20例の検討ではその80%にHTLV-I関連反応が認められたとの報告がある¹⁶⁾。組織学的には、細気管支粘膜下から肺胞中隔にかけてリンパ球浸潤を認め、肺胞壁内には泡沫細胞集簇を伴う(図15)。これらのリンパ球はほとんどが異型のないT細胞であり、形態上はDPBとの鑑別は困難である。なお、泡沫細胞集簇については見解が分かっている。HTLV-I感染者は南九州に多く、患者の出生地などを確認し、血清学的検索およびHTLV-I遺伝子の検索が望ましい。

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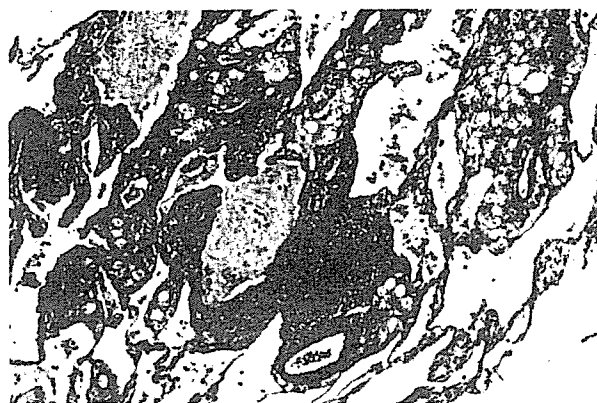


図15 HABAの組織像 呼吸細気管支壁にリンパ球浸潤を認め、周囲の肺胞壁内には泡沫細胞集簇を伴っている。

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Mutant murine leukemia virus Gag proteins lacking proline at the N-terminus of the capsid domain block infectivity in virions containing wild-type Gag

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Abstract

We have investigated the properties of murine leukemia virus Gag mutants in which the p12-CA cleavage site is altered. In one mutant, the cleavage is blocked; in the other, the conserved proline at the N-terminus of CA has been replaced with glycine. No infectivity was detected in either mutant. Mutant particles cannot synthesize full-length DNA upon infecting permissive cells. Particles composed of a mixture of wild-type and mutant proteins have severely impaired infectivity. These mixed particles are defective in their ability to synthesize DNA upon infection, but this defect is less severe than the loss of infectivity. Thus, proteins lacking the correct N-terminus of CA inhibit DNA synthesis and also interfere with formation or integration of a full-length, normal provirus. The results imply that CA proteins function as part of a large, highly organized structure in reverse transcription and apparently at a later step as well.

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Keywords: Retroviruses; Capsid proteins; Infectivity; Reverse transcription; Dominant negative; Trans-dominant; Phenotypic mixing; Virus maturation

Introduction

The principal structural component of a retrovirus particle is the virus-coded Gag protein. After the particle is released from the virus-producing cell, Gag is cleaved by the viral protease (PR) into a series of cleavage products, always including (from N- to C-terminus) matrix (MA), capsid (CA), and nucleocapsid (NC). This series of cleavage events is termed “maturation” of the particle and is essential for infectivity (Swanstrom and Wills, 1997).

The structure of the CA protein of HIV-1 has been investigated in detail, using both nuclear magnetic resonance and X-ray crystallography. Retroviral CA proteins are composed of two domains connected by a flexible linker. Their N-terminal

residue is always proline. Following the cleavage event that generates this N-terminus, the first ~50 amino acids of CA fold into a β -hairpin, and the proline forms a buried salt bridge with an internal aspartate residue (Gamble et al., 1996; Gitti et al., 1996; Tang et al., 2002).

Maturation entails a drastic change in the morphology of the virion. One of the hallmarks of this change is the appearance of the “mature core” of the particle, a densely staining body in the interior of the virion. Recent studies on structures formed *in vitro* by purified CA proteins of HIV-1 and murine leukemia virus (MLV) show that the mature core is composed of CA molecules. Within this core, CA proteins are evidently arranged in a lattice in which the N-terminal domains of groups of six CA molecules are in hexameric rings; each ring is joined to neighboring rings by dimeric contacts between C-terminal domains (Ganser et al., 2003; Li et al., 2000; Mortuza et al., 2004). The planar hexagonal lattice is closed by the presence of twelve pentameric “defects” (Ganser et al., 1999).

Several types of evidence suggest that the correct mature core structure is essential for infectivity, and that this structure depends in turn upon the presence of the proline at the N-

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terminus of CA. Thus, mutants in which the proline is replaced by another amino acid (Fitzon et al., 2000) (see below) or in which the release of CA from Gag is inhibited by a change in the cleavage site (Gottlinger et al., 1989; Oshima et al., 2004) are not infectious. Similar results are also observed when the aspartate partner in the salt bridge in HIV-1 CA is replaced by alanine (Tang et al., 2003).

In the present work, we have analyzed the properties of MLV mutants at the N-terminus of CA. We have also studied MLV particles containing a mixture of mutant and wild-type proteins. The mutants investigated here included one in which maturation cleavage at the N-terminus of CA is blocked by a change in the residue preceding proline in Gag and one in which the N-terminal proline in CA was replaced by glycine. We found that both of these mutants potentially inhibit the functions of the wild-type, reducing the specific infectivity of the mixed particles. The data indicate that these mutant proteins interfere with the ability of the particle to synthesize viral DNA upon entering the new host cell. The results support the hypothesis that an organized assemblage of CA molecules in the mature particle plays a crucial role in facilitating reverse transcription; this functional complex is evidently disrupted by the presence of mutant proteins in the particle, even when wild-type proteins are in excess. However, the inhibition of DNA synthesis did not appear to be sufficient to explain the loss of infectivity in the mixed particles. Thus, the data further suggest that the correct structure of the mature core is also required for the correct formation of the final DNA product or for a subsequent step, such as integration of the DNA into the chromosome of the host cell.

Results

Properties of "S2G"

The MLV Gag protein, Pr65, is cleaved into MA, p12, CA, and NC during virus maturation, as indicated in Fig. 1A. We

have previously described mutants at cleavage site 2 ("S2"), the p12-CA cleavage site (Oshima et al., 2004). We found that replacement of the tyrosine at the C-terminus of p12 (the P1 position of S2) with a charged residue, such as aspartate, completely blocked cleavage at this site. This mutant was designated S2D. The location of this mutant in the Gag protein is illustrated schematically in Fig. 1A. We also observed that cleavage at the other two sites of maturation cleavage, particularly the CA-NC cleavage site, was somewhat inefficient in S2D particles (Oshima et al., 2004). In the present work, we have also investigated a mutant in which the proline at the N-terminus of CA (i.e., the P1' position of site 2) was replaced by glycine; we refer to this mutant as "S2G" for convenience.

Fig. 1B shows an analysis of S2G particles, using immunoblotting with anti-CA antiserum. Also included in the figure, for comparison, are particles of S2D (in which there is no free CA, but only the 42-kDa p12-CA fusion protein (Oshima et al., 2004)), as well as wild-type and PR⁻ particles. It can be seen that the S2G profile contains a substantial amount of CA protein. There is also a significant level of a protein migrating just slightly faster than the 42-kDa protein of S2D; this is p40, the CA-NC fusion protein, since it reacts with anti-NC as well as anti-CA antisera (data not shown). p40 was previously observed in S3R particles, in which cleavage between CA and NC was blocked (Oshima et al., 2004) (see Fig. 1A). Thus, despite the fact that this mutant is altered at the N-terminus of CA, it exhibits efficient cleavage at the N-terminus, but only partial cleavage at the C-terminus, of CA. Both S2G and S2D particles also contain two larger intermediates, presumably MA-p12-CA and p12-CA-NC.

The morphology of S2G particles was also examined. Released virions were collected for thin-section electron microscopy by immunoprecipitation with anti-SU antibody as described (Oshima et al., 2004). We found (Fig. 2) that they are similar to wild-type MLV particles, but that their structure is considerably more irregular. For example, many particles contain condensed material in their interiors but lack a clearly defined core. Other particles resemble immature particles, but

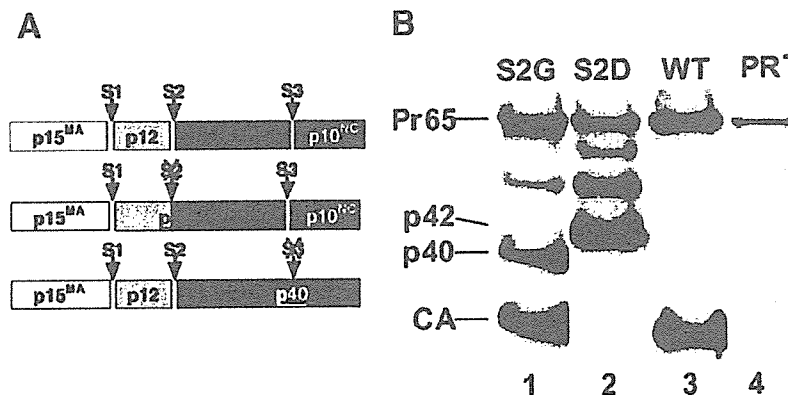


Fig. 1. Cleavage of MLV Gag and CA-containing protein species in S2G virions. (A) (top row) Scheme showing normal cleavage of MLV Gag into MA, p12, CA, and NC; (middle row) production of p42 in mutants such as S2D which block cleavage at site 2 (between p12 and CA); (bottom row) production of p40 when cleavage at site 3 is blocked. (B) Particles of S2G (lane 1), S2D (lane 2), wild-type MLV (lane 3), and PR⁻ MLV (lane 4) were collected from culture fluid and analyzed by immunoblotting with anti-CA antiserum. Mobilities of Pr65^{Gag}, p30^{CA}, and the p42 and p40 cleavage intermediates are indicated.

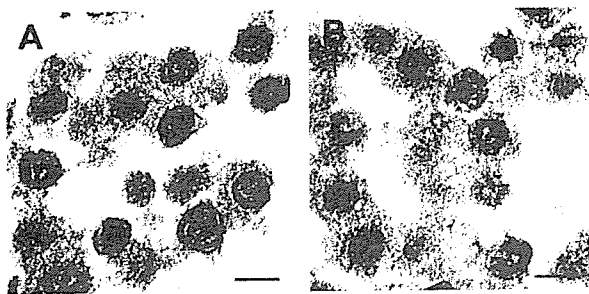


Fig. 2. Morphology of S2G virions. Virions were collected on protein G-Sepharose beads as described (Campbell et al., 2002). (A) Wild-type MLV. (B) S2G. Scale bars: 100 nm.

the ring of darkly staining material under the membrane of the particle is incomplete. Examples of these morphologies are in the field shown in Fig. 2B.

We also measured infectivity of S2G particles, using either a luciferase reporter or the green fluorescent protein (GFP)-based system described earlier (Oshima et al., 2004). No infectivity was detected in either of these assays, since the luciferase or GFP activity induced by the mutant particles was no higher than in cultures “mock-infected” using culture fluids from cells transfected with the empty vector pGCcos3neo; the luciferase data are shown in Table 1. The specific infectivity of these particles is at least several hundred-fold below that of wild-type particles.

We have previously analyzed the genomic RNA in S2D mutant particles (Oshima et al., 2004). These tests gave no suggestion that packaging of the RNA was reduced in the S2D particles. However, it was conceivable that packaging is defective in S2G particles. We therefore produced S2G particles, along with wild-type control particles, in 293T cells stably transfected with the MLV-derived vector pLXSH (Miller et al., 1993) (see below). We analyzed the particles for hygromycin phosphotransferase (hph) RNA by real-time RT-PCR. No significant differences between hph RNA contents of S2G and wild-type (or S2D) virions were observed (data not shown).

Properties of virions containing a mixture of wild-type and S2D or S2G Gag proteins

The focus of the present study was the biological properties of MLV particles composed of a mixture of wild-type and

mutant Gag proteins. We measured infectivity by co-transfecting 293T cells with an MLV-based reporter vector along with the MLV proviral clones being analyzed.

A series of 293T cultures was transfected with constant amounts of reporter vectors and wild-type MLV plasmid together with graded amounts of S2D or S2G mutant plasmid. Virions in the culture fluid of the transfected cells were collected and assayed for their Gag protein profile and their infectivity. Immunoblots are shown in Fig. 3. The only proteins detected with anti-p30^{CA} antiserum in the wild-type particles (lanes 1–4) are p30^{CA} and a small amount of the uncleaved precursor, Pr65^{Gag}. As noted above, however, the S2D clone (lane 10) gives rise to virions that contain p42 (the p12-CA fusion protein) and two larger intermediates in addition to Pr65^{Gag} (Oshima et al., 2004). When increasing amounts of S2D plasmid are added to the wild-type plasmid (lanes 5–9), these same species, as well as free CA, are observed. A culture transfected with equal amounts of the two plasmids (lane 7) produces particles containing approximately equal amounts of CA and p42. Thus, neither of the MLV plasmids affects the contribution of the other plasmid to the virions produced by the co-transfected cells (It is interesting to note one deviation from this simple statement. There is more free CA in lane 9 than in lanes 5–8, despite the fact that CA is only produced from the wild-type plasmid, which is at a constant level in lanes 5–9. It seems likely that virus assembly is cooperative, so that the expression of S2D Gag increases virion production and, consequently, the export of wild-type Gag in virus particles.) Similar results were obtained in the cultures co-transfected with wild-type plasmid and increasing amounts of S2G (lanes 12–17).

Infectivity of mixed virus stocks like those shown in lanes 5–9 and 12–16 was assessed by infecting 293T cells expressing MCAT (Albritton et al., 1989) with them and assaying the infected cultures 48 h later for luciferase activity. Normalized results of these assays are shown in Fig. 4. It is evident that the infectivity values are substantially lower in the mixed particles than in the wild-type controls; for example, particles with equal amounts of wild-type and S2D protein have a titer several hundred-fold below that of the wild-type control. Similar results have also been obtained in assays on NIH3T3 cells (data not shown). (In the experiment presented here, S2D was more detrimental in its effects on infectivity than S2G. However, in some experiments, S2G has been fully as deleterious as S2D.

Table 1
Biological activity of MLV particles containing S2D or S2G Gag

Sample	LUC	Protein, µg/ml	Hph copies	CCR5 copies	Hph/CCR5
WT	30,876 ± 1344	370	16,472 ± 4664	36,641 ± 2289	0.449
S2D	37 ± 6	395	0.9 ± 1.8	73,530 ± 7035	0.00001
S2G	43 ± 12	483	4.6 ± 5.3	89,833 ± 13,016	0.00005
RT ⁻	N.D.	363	14.8 ± 9.8	80,719 ± 16,743	0.0002
pGCcos3neo	30 ± 0	341	8.5 ± 5.7	100,117 ± 13,844	0.00008

Virus particles were produced by transfecting pLXSH-containing cells with 4 µg of wild-type or mutant MLV plasmid. Each plate also received 4 µg of pBabe-Luc DNA. Infectivity was assessed by measuring luciferase activity in extracts of 293T/MCAT cells infected by the particles, while the amount of hph DNA in the infected cells was used to measure reverse transcription. The single-copy cellular gene CCR5 was used as a DNA recovery control. Luciferase activity (“LUC”) is expressed as relative light units per 20 µl of cell extract. N.D., not determined.

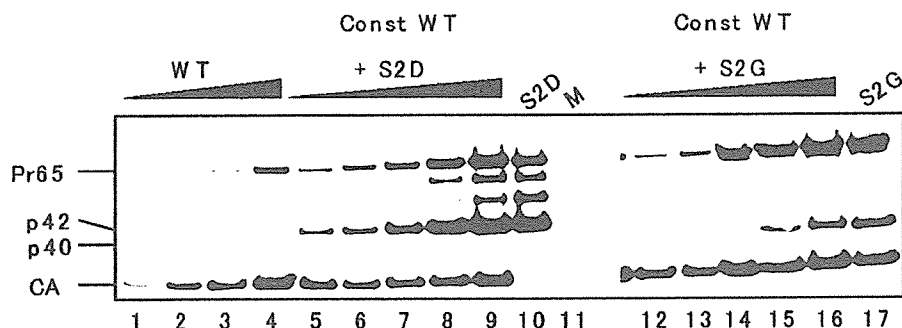


Fig. 3. Composition of virions produced following co-transfection of mutant and wild-type MLV plasmids. 10-cm dishes of 293T cells were transfected with 1 (lane 1), 2 (lane 2), 4 (lane 3), or 8 (lane 4) μ g of wild-type plasmid, or with 4 μ g of wild-type plasmid + 1 (lane 5), 2 (lane 6), 4 (lane 7), 8 (lane 8), or 12 (lane 9) μ g of S2D plasmid, or with 12 μ g S2D (lane 10) or pGCcos3neo (lane 11) plasmid, or with 4 μ g wild-type plasmid + 1 (lane 12), 2 (lane 13), 4 (lane 14), 8 (lane 15), or 12 (lane 16) μ g S2G plasmid, or 12 μ g S2G plasmid (lane 17). All plates also received 4 μ g each of pBabe-Luc and pLZRS-EGFP DNAs. The plasmid vector pGCcos3neo was included in the transfections as needed to maintain the total amount of DNA added to each plate at 24 μ g. Virions produced by the transfected cultures were analyzed by immunoblotting with anti-CA antiserum.

We have been unable to determine the reason for this variation between experiments).

It seemed possible that these results are due to some peculiarity of the luciferase reporter system used here. To test this possibility, we included pLZRS-EGFP, as well as pBabe-Luc, in the co-transfections with mixtures of mutant and wild-type plasmids and assayed the culture fluids for the titer of GFP-inducing infectious units as well as for the infectivity of the luciferase vector. The relative titers obtained with the two reporter systems were virtually superimposable (data not shown). Thus, the reduced infectivity of mixed particles containing S2D or S2G protein is evidently independent of the assay used and presumably reflects an intrinsic property of these virions.

As noted above, S2G particles are inefficiently cleaved at the C-terminus of CA, so that they give rise to a significant amount of the p40 CA-NC fusion protein (Figs. 1B, 3). It seemed possible that this property was responsible for the ability of S2G Gag to reduce the infectivity of mixed particles

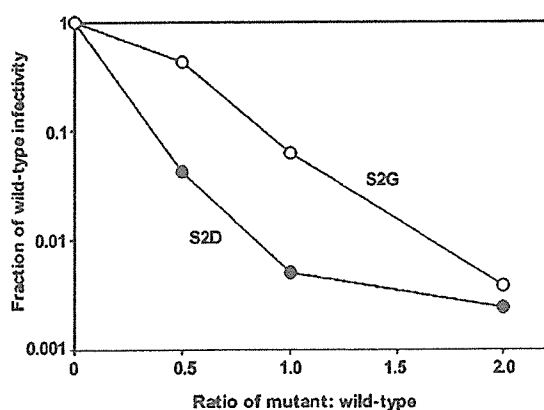


Fig. 4. Effects of S2D and S2G plasmids on infectivity of mixed particles. Virions were produced as in Fig. 3. Luciferase activities (corrected for small differences in protein concentration in extracts of infected cells) were divided by the activity obtained using 4 μ g of wild-type plasmid + 12 μ g of pGCcos3neo DNA. Closed circles, S2D; open circles, S2G.

(Fig. 4). To test this hypothesis, we also assessed the infectivity of particles produced following co-transfection of wild-type and S3R MLV; the leucine at the C-terminus of CA has been replaced in S3R with arginine, completely preventing cleavage between CA and NC (Oshima et al., 2004). The results of these tests are shown in Fig. 5. As can be seen in Fig. 5B, the admixture of S3R Gag protein (shown in Fig. 5A) had almost no effect on the infectivity of the particles. Thus, the inhibition of wild-type infectivity by the S2G Gag protein is probably not due to the inefficiency of cleavage at site 3.

DNA synthesis upon infection with particles containing both wild-type and mutant Gag proteins

It was of interest to determine whether the presence of the S2D or S2G proteins in the mixed particles interfered with the ability of the particles to synthesize viral DNA upon entry into susceptible cells. We therefore measured reverse transcription products, using real-time PCR, at 24 h after infection with these particles. One potential technical problem in these experiments is the presence in the virus preparations of plasmid DNA from the transient transfection used to produce the particles. To eliminate this background, we adopted the following strategy. 293T cells were first stably transfected with the MLV-derived vector pLXSH, which contains the coding sequence for hph (Miller et al., 1993). The wild-type and mutant MLV clones (together with pBabe-Luc, which enabled us to conveniently measure the infectivity of the progeny viruses) were then co-transfected into these hygromycin-resistant cells, and virus produced following this transient transfection was finally analyzed for its ability to synthesize hph DNA following infection of permissive cells as described in Materials and methods. Cells were lysed 24 h after infection and assayed for hph DNA by real-time PCR. It should be noted that hph DNA synthesis depends upon completion of the preceding steps in reverse transcription, i.e., (–) strand strong-stop DNA synthesis and the first strand transfer event. To control for possible variation in recovery of DNA from the infected cells, we also

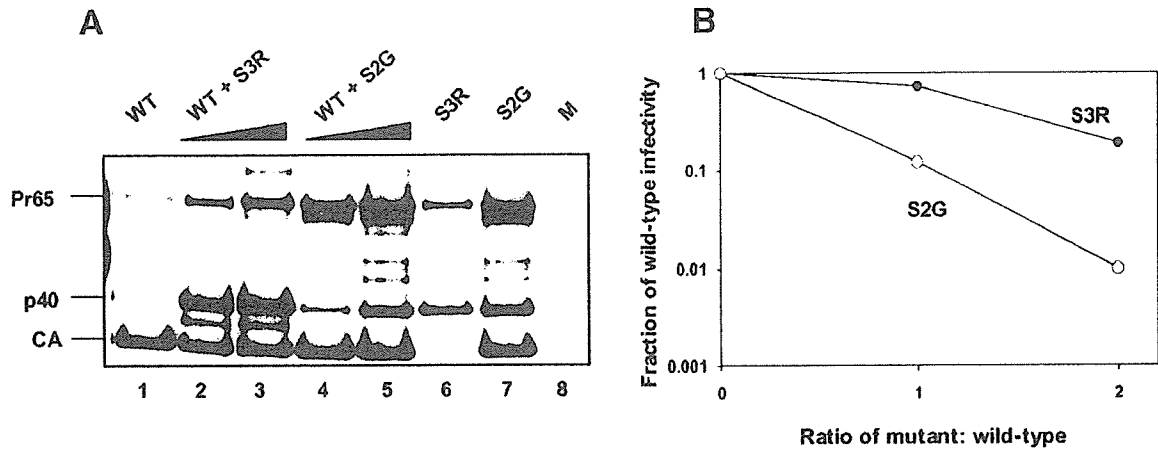


Fig. 5. Properties of virions produced following co-transfection of wild-type and S3R plasmid DNA. 10-cm dishes of 293T cells were transfected with 4 μ g of wild-type plasmid + 4 or 8 μ g of S3R or S2G plasmid. Control cultures were transfected with 4 μ g of wild-type or mutant plasmids alone. All cultures also received 4 μ g of pBabe-Luc DNA. Total plasmid DNA in the transfections was kept constant by addition of pGCcos3neo DNA. (A) Protein composition of the virions from the transfected cells, as analyzed by immunoblotting with anti-CA antiserum; (B) infectivity of the virions, assayed by infecting NIH3T3 cells and measuring luciferase activity 48 h later. Luciferase values were divided by the activity obtained with 4 μ g wild-type plasmid + 8 μ g pGCcos3neo DNA. Closed circles: effect of S3R; open circles: effect of S2G.

measured the amount of the single-copy gene CCR5 in our cell extracts. The variation among all samples with respect to CCR5 content was less than 3-fold (data not shown).

We found (Table 1) that particles containing only S2D or S2G mutant proteins were incapable of synthesizing hph DNA; that is, the corrected values of “hph DNA” in the infected cells were always at least 5000-fold lower than in the wild-type controls and were never significantly higher than those in mock-infected control cells. Thus, cleavage at the N-terminus of CA is apparently essential for reverse transcription of the full-length viral genome, and replacement of the conserved proline residue with glycine at this site is also incompatible with synthesis of the entire DNA product. We then measured hph DNA synthesized in cells infected with the virions containing mixtures of wild-type and S2D or S2G proteins. Results

(following normalization to the wild-type control) are shown in the closed circles in Fig. 6. It is obvious that as the proportion of mutant protein in the virion increases, the particle becomes progressively impaired in its ability to perform reverse transcription. It is striking to note, however, that the inhibition of viral infectivity induced by the presence of the mutant proteins (dashed lines) is, in general, significantly greater than the inhibition of hph DNA synthesis (closed circles). In other words, many particles which synthesize DNA in newly infected cells still fail to establish infection.

In an effort to further define the block in infection imposed by the presence of Gag proteins with alterations at the N-terminus of CA, we also measured the level of hph DNA in cells that had been passaged for 11 days following infection. As shown in the open circles in Fig. 6, the relative levels of

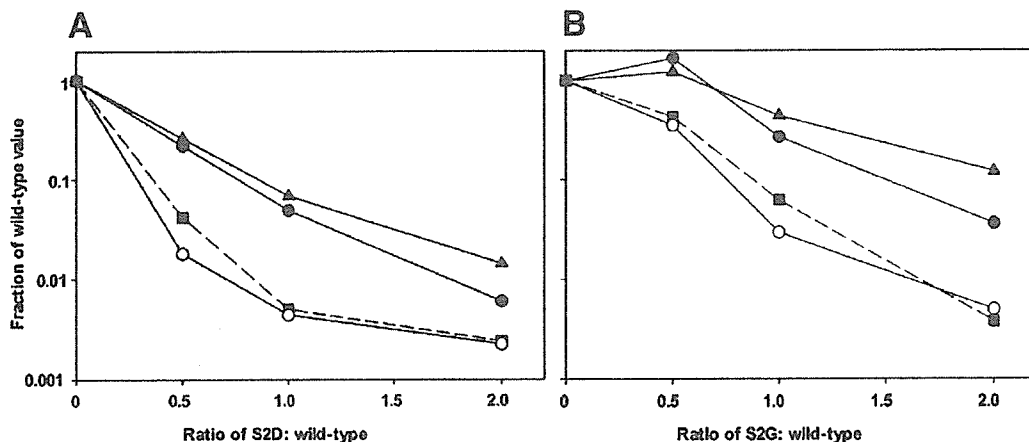


Fig. 6. Effect of S2D (A) and S2G (B) on infectivity of and DNA synthesis by MLV particles. 293T cells expressing MCAT were infected with the virions analyzed in Fig. 3 and were lysed 24 h later for assay of hph DNA (closed circles) and for 2-LTR circular DNA (triangles). Replicate plates were lysed 48 h later for luciferase activity (dashed lines; data as in Fig. 4). Replicate cultures were also passaged for 11 days and then lysed and assayed for hph DNA (open circles). All DNA measurements were also corrected for differences in recovery of CCR5 DNA.