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Immunoglobulin synthesis in primary and myeloma amyloidosis

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SUMMARY

Bone marrow cells from 14 patients with primary amyloidosis and two patients with myeloma amyloidosis were studied by immunofluorescence and biosynthesis experiments after incorporation of radioactive amino acids. Cells from four patients affected with non-myeloma secondary amyloidosis were also studied as controls. In primary amyloidosis, monoclonal plasma cell populations were demonstrated by immunofluorescence in virtually every case, even in patients without serum and urine monoclonal immunoglobulin and with a normal percentage of bone marrow plasma cells. Biosynthesis experiments showed the secretion of large amounts of free light chains, most often of the λ type, in every primary or myeloma amyloidosis case and the presence of light chain fragments in almost all cases. Special features in certain patients were the synthesis of short γ chains (two cases), assembly block at the HL half molecule level of a monoclonal IgA (one case) and secretion of decameric abnormally large κ chains (one case). This is in contrast with non-myelomatous secondary amyloidosis where the distribution of bone marrow plasma cells was normal by immunofluorescence and where normal sized immunoglobulins were synthesized, without free light chain secretion and fragments. These data confirm that primary amyloidosis belongs to plasma cell dyscrasias and emphasize the role of free light chains and light chain fragments in the pathogenesis of amyloid deposition.

Keywords immunoglobulin synthesis primary amyloidosis myeloma immunofluorescence

INTRODUCTION

Tissue deposition of monoclonal immunoglobulin (Ig) (mostly light chain)-derived material is a major complication of the various syndromes of plasma cell dyscrasia, whatever their haematological presentation and apparently benign or overtly malignant nature. Two major conditions account for such depositions: light chain deposition disease (LCDD, Randall *et al.*, 1976) which would be more appropriately named monoclonal immunoglobulin deposition disease since tissue deposits include determinants of both a heavy and a light Ig chain in certain patients (Preud'homme *et al.*, 1980c; 1982) and AL type amyloidosis. Our immunofluorescence study in 22 patients with LCDD showed a monoclonal population of bone marrow plasma cells in every case, irrespective of the presence or absence (about one-third of cases) of serum and/or urinary monoclonal Ig (Preud'homme, 1988). The study of Ig biosynthesis disclosed two apparently distinct situations in LCDD: (1) in about two-thirds of the patients studied, Ig chains were grossly abnormal. For light chains, there was a triple abnormality: size (abnormally short or enlarged chains), glycosylation (the carbohydrate content of κ chains was 15% and 11% in the two patients in whom enlarged light chains could be purified) and polymerization *in vivo* and *in vitro* by covalent and non-covalent bonding. When heavy chains were deposited, they were abnormally short and did not fully assemble with light chains. (2) In other cases, monoclonal Ig were normal-sized and light chain were unglycosylated but they also displayed a strong tendency to polymerize (Preud'homme *et al.*, 1980b, c; 1982; Gallo *et al.*, 1980; 1982; Ganeval *et al.*, 1982; 1984; Preud'homme, 1988). One of the reasons that led us to search for Ig biosynthesis abnormalities in LCDD was the occurrence of this deposition syndrome in the so-called nonsecretory (i.e. without detectable serum and urine monoclonal Ig) myeloma. Indeed, plasma cells from most patients with non-secretory myeloma secrete structurally abnormal Ig molecules that are not found in serum and urines (Preud'homme *et al.*, 1976; 1977a. Amyloidosis also occurs in non-secretory myeloma (Azar *et al.*, 1972; Preud'homme *et al.*, 1976a). This and the concept introduced by Osserman, Takatsuki & Talal (1964) that primary amyloidosis is probably a plasma cell dyscrasia prompted us to examine by cytoplasmic immunofluorescence and biosynthesis experiments bone marrow cells from patients with primary and myeloma

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Table 1. Secondary amyloidosis.

Patient no.	Underlying disease	Monoclonal Ig in serum or urine	Ig containing bone marrow cells (%)								Biosynthesis experiment
			γ	α	μ	δ	κ	λ	ΣH	ΣL	
1	Portuguese amyloidosis	None	0·4*	0·1	0·1	0	0·4*	0·4*	1	1	Normal
2	Psoriasis, ankylosing spondylitis	None	0·64	0·44	0·14	0	0·64	0·45	1·22	1·09	—†
3	Behcet	None	0·56	0·33	0·30	0	0·78	0·33	1·19	1·11	Normal
4	Kidney carcinoma	None	0·74	0·34	0·11	0	0·79	0·33	1·19	1·12	Normal

* Accurate counting not possible due to the lack of a sufficient number of cells.
† Incorporated counts too low for an interpretable study.

amyloidosis. Cells from patients with non-myelomatous secondary amyloidosis were studied as controls.

MATERIALS AND METHODS

Patients

Bone marrow cells were obtained at the time of diagnosis and before any specific therapy. They were examined immediately and the same samples were used for both immunofluorescence and Ig synthesis study. Patients under study included 14 patients with primary amyloidosis, i.e. without any geographical, familial and pathological context compatible with secondary amyloidosis and with no evidence of associated disease including myeloma in spite of careful haematological evaluation. In two of these patients, a diagnosis of myeloma was considered as probable later upon follow-up. No monoclonal Ig was detectable by electrophoretic and immunoelectrophoretic analysis of serum and concentrated urines in four cases, small amounts of urinary Bence-Jones protein (BJ) were found in four patients and the serum from six patients contained a monoclonal Ig in moderate concentrations (four IgG, one IgA of the α1 subclass and one IgM). In five further primary amyloidosis patients, the bone marrow sample was poor and only the Ig biosynthesis study was performed, but incorporated counts were too low for interpretable results. Two patients with myeloma and amyloidosis were also studied, one affected with non-secretory myeloma and one with BJ myeloma. The four patients with secondary amyloidosis whose cells were studied as controls were affected with Portuguese amyloidosis, psoriasis and ankylosing spondylitis, Behcet's syndrome and kidney carcinoma, respectively.

Immunofluorescence

Bone marrow cells obtained by aspiration were studied by cytoplasmic immunofluorescence with monospecific conjugates against the various human heavy (H) and light (L) Ig chains. The method used to detect cellular Ig, the preparation, characteristics and specificity controls of the conjugated $F(ab')_2$ fragments anti-human Ig have been described in detail previously (Preud'homme & Labaume, 1976). It should be pointed out that every anti-heavy and anti-light chain conjugate is a pool of several antisera obtained after immunization with either entire Ig molecules or isolated chains. Anti-heavy chain sera were absorbed on insolubilized relevant Ig to remove anti-V_H antibodies and this was controlled by several experiments. In contrast, anti-light chain sera were absorbed on various proteins including entire Ig and and free light chains of the other type and the controls showed their specificity with respect to κ or λ determinants. Hence, they are likely to contain antibodies directed to both the variable and constant regions of the light chains. When needed for an accurate counting (i.e. in the primary and non-myelomatous secondary amyloidosis patients in whom the total percentage of bone marrow plasma cells was in the normal range), 5,000 to 10,000 bone marrow cells by IF slide were counted.

Biosynthesis experiments

Ig biosynthesis was studied as previously described (Preud'homme *et al.*, 1975; 1977a, b; 1980a, b). Briefly, bone marrow cells were washed in spinner minimal essential medium lacking valine, threonine and leucine and incubated in the same medium for 3 h with ^{14}C or ^{3}H valine, threonine and leucine. Supernatants containing secreted proteins (sec.) were separated by centrifugation and cell pellets were lysed by the non-ionic detergent NP40. Cytoplasmic extract (cyto.) and sec. were immunoprecipitated using polyvalent anti-human Ig rabbit $F(ab')_2$ fragments and goat anti-rabbit Ig antibodies. Washed precipitates were dissolved in sodium dodecyl sulphate (SDS) and analysed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) at various gel concentrations. Internally labelled IgG, H and L chains from the murine plasmacytoma MPC 11 were used as molecular weight (M_w) markers.

RESULTS

Non-myelomatous secondary amyloidosis

In the four studied patients with secondary amyloidosis, IF study of bone marrow cells showed a normal distribution of Ig containing cells both for H ($\gamma > \alpha > \mu > \delta$) and L ($\kappa > \lambda$) chains. The sum of H chain containing cells equalled that of L chain producing plasma cells (Table 1). Ig biosynthesis study showed the production of normal Ig molecules. Free L chains were found in cytoplasmic extracts, which is normal (there is normally a pool of cytoplasmic free L chains), but not in secretions (Fig. 1, top).

Primary amyloidosis and myeloma amyloidosis

In this group of patients, IF study showed the presence of a monoclonal plasma cell population most often of the λ type in

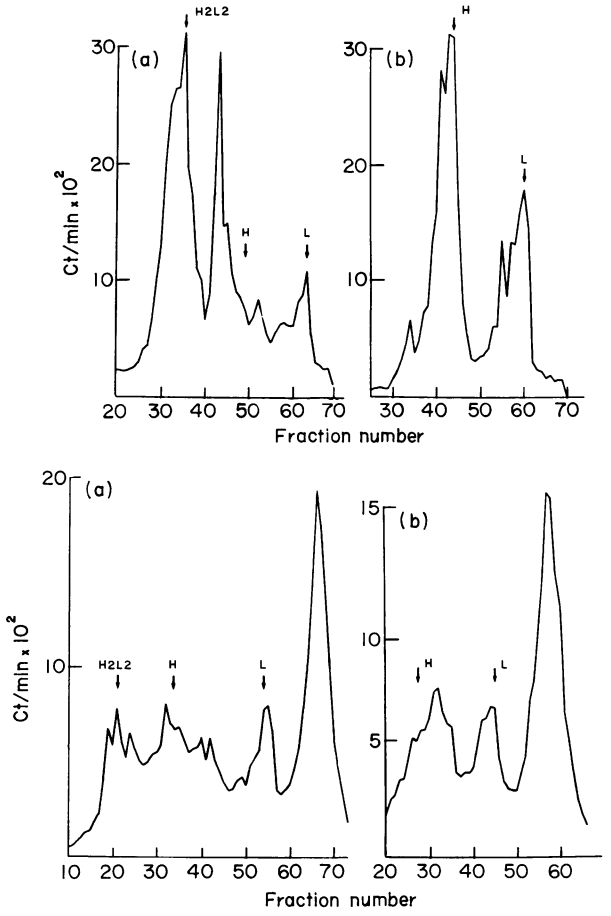

Fig. 1. Comparison of SDS-PAGE analysis of ^{14}C-labelled cytoplasmic extracts from two bone marrow cell suspensions containing a normal percentage of plasma cells. The upper panel shows results in the non-myeloma secondary amyloidosis patient No. 3. The lower panel displays immune precipitates from primary amyloidosis patient 3′ bone marrow cells. Cylindrical gels with ^{3}H-internally labelled IgG (H2L2), heavy (H) and light (L) chains from the murine plasmacytoma MPC 11 as internal markers in the gels. Position of the markers is indicated by arrows. (a) unreduced immune precipitates (5% acrylamide gels); (b) reduced and alkylated immune precipitates (7·5% acrylamide gels). On the upper panel, note the two shoulders in the heavy chain peak on reduced gels; they probably correspond to μ and α chains. Similarly, the shoulder in the H2L2 peak (unreduced gels) is probably IgA (IgM does not enter 5% gels) and the one in the light chain peak is probably due to λ chains some of which migrate more slowly than κ chains on SDS-gels. On the lower panel, note the 12 kD fragment which is the major peak. This fragment was also present in secretion, together with free light chains and normal-sized assembled molecules. This is especially striking in view of the very small excess of λ containing plasma cells by immunofluorescence (Table 2).

every case but two, even when the percentage of plasma cells amongst bone marrow cells was normal (Table 2). These data are summarized in Table 3. Interpretable results of biosynthesis experiments were available in 10 cases (Table 2). The secretion of large amounts of free L chains was a constant feature in all patients, including those without detectable urinary BJ protein and Patient No. 6 in whom no monoclonal plasma cell population could be detected by IF. These L chains were secreted as monomers and, predominantly, dimers (except in Patient 10). The apparent mol. wt. of λ chains was large (25–27 kD) in five cases. This is meaningless since it is well known that certain human λ chains have an abnormal behaviour in SDS-gels, leading to an erroneous appreciation of their mol. wt. The apparent mol. wt of the L chains was identical in cytoplasm and secretion in every case. One or two fragments of the size of about a half-L chain or slightly larger were present in cytoplasmic extracts from nine of 10 patients but detectable in significant amounts in the secretion in only three cases. Interestingly, such fragments were the major cytoplasmic anti-Ig precipitable component in the two cases (Nos. 2 and 3) with no detectable monoclonal Ig or BJ in serum and urine (Fig. 1, bottom).

Four primary amyloidosis patients had urinary BJ λ. In one case (No. 6) a huge excess of free L chain was found besides normal Ig molecules. This is interesting since although this patient had small amounts of urinary λ type BJ proteins, no monoclonal plasma cell population could be detected by immunofluorescence. However, the major Ig species in gels were free light chains that were predominantly secreted as dimers. There was also a cytoplasmic fragment that was barely detectable in secretion. In Patients 7 and 8, only L chains (secreted as dimers) and fragments were visible on the gels. In Patient 9, in whom a monoclonal IgG λ was found by bone marrow IF, the cells secreted L chains, L chain dimers and fragments together with assembled molecules. Upon reduction and alkylation, the major H chain component was abnormally short (apparent mol. wt 48 kD). Patient 10 had a BJ κ myeloma. His bone marrow plasma cells produced large (31 kD) κ chains that were secreted as a single covalent molecule of about 300 kD (probable decamer) (Fig. 2, top).

Results of biosynthesis experiments are available in three of the six primary amyloidosis patients whose serum contained an entire monoclonal Ig. As in the other cases, free L chains were secreted, mostly as dimers. One of these patients (No. 12) is the only one in whom we did not detect L chain fragments. His monoclonal IgG was made of an abnormally short H chain (apparent mol. wt 47 kD). The serum of Patient 15 contained an IgAλ of the IgA1 subclass. His plasma cells synthesized normal size α chains (apparent mol. wt 60 kD) that assembled with L chains into a HL half molecule, with virtually no peak of the size of H2L2 molecules (Fig. 2, bottom). Materials was not available in sufficient amounts for a biochemical study of the serum M component.

DISCUSSION

The present study of cells from 14 patients with primary amyloidosis (and two patients with myeloma-associated amyloidosis) confirms Osserman's concept that this syndrome belongs to plasma cell dyscrasias. Indeed, evidence for monoclonal plasma cells was obtained by bone marrow IF in all patients but two, even in those with normal percentages of bone marrow plasma cells and without detectable monoclonal Ig in serum and urine. Furthermore, in the patient (No. 6) with a normal distribution of plasma cells by IF whose bone marrow cells could be studied for Ig biosynthesis, the amyloid substance stained for λ determinants, small amounts of BJ λ were present in urines and bone marrow cells secreted a huge excess of free L chains. It is worth noting that in both LCDD (Preud'homme *et al.*, 1980b, c; 1982; Ganeval *et al.*, 1982; 1984) and amyloidosis, certain patients present with nonsecretory myeloma or plasma

Table 2. Primary amyloidosis and myeloma amyloidosis.

Patient no.	Diagnosis	Monoclonal Ig in serum or urine	Ig determinants in amyloid deposits by IF	Ig containing bone marrow cells (%)								Free L chain secretion	Mol. wt*	Ig biosynthesis experiments			Comments
														Fragments			
				γ	α	μ	δ	κ	λ	ΣH	ΣL			cyto.	mol. wt	sec.	
1	Primary amyloidosis	None	ND	*6*	1	1	ND	1	*6*	*7*	*7*				ND		
2	Primary amyloidosis	None	ND	0·36	0·11	0·17	0·02	0·45	*1·0*	0·66	*1·75*	L2>L	22	+++	11,9	0	F as the major cytoplasmic Ig
3	Primary amyloidosis	None	All chains	0·07	0·13	0·10	0	0·39	*0·32*	0·30	*0·71*	L2>L	22·5	+++	12	++	F as the major cytoplasmic Ig
4	Primary amyloidosis	None	ND	0·22	0·05	0·14	0·01	0·25	0·13	0·42	0·38				ND		
5	Myeloma	None	ND	1	*19*	1	ND	1	*19*	*19*	*19*				ND		
6	Primary amyloidosis	BJ λ	λ	0·18	0·07	0·04	0	0·17	0·09	0·29	0·26	L2≫L	27	++	13	±	
7	Primary amyloidosis†	BJ λ	None	0·1	0·1	0·1	0	0·1	*3·5*	0·1	*3·5*	L2	27	+	12	0	No detectable H chains
8	Primary amyloidosis	BJ λ	ND	2	0·5	0·5	ND	2	*6*	3	*8*	L2	22·5	+	18,11	0	No detectable H chains
9	Primary amyloidosis	BJ λ	ND	*3*	1	0·5	ND	1·5	*3*	*4·5*	*4·5*	L2≫L	26	+	20,17	++	Abnormally short H chains
10	Myeloma	BJ κ	ND	1	1	1	1	*30*	1	1	*30*	L10	31	+	15	0	Abormally large κ chains secreted as a decamer
11	Primary amyloidosis	IgG λ	ND	*4*	0·1	0·1	0	0·1	*4*	*4*	*4*				ND		
12	Primary amyloidosis	IgG λ	λ				ND					L2	26	0		0	Abnormally short H chains
13	Primary amyloidosis	IgG κ	ND	*1*	0	0	0	*1*	0	*1*	*1*				Uninterpretable‡		
14	Primary amyloidosis	IgG κ	ND	0·23	0·09	0·06	0	*0·42*	0·07	0·38	*0·49*	L2>L	22·5	++	12	+	
15	Primary amyloidosis†	IgA1 λ	None	0·86	*2·55*	0·10	0	0·40	*2·35*	3·5	2·75	L2>L	25	++	13	0	Assembly block of IgA molecules
16	Primary amyloidosis	IgM λ	λ	0·5	1	*9*	ND	1·5	*7*	10·5	8·5				Uninterpretable‡		

* Apparent mol. wt of the chain monomer on gels (kD)

† Probable diagnosis of myeloma considered upon follow-up.

‡ Incorporated counts too low.

ND, not cone.

Table 3. Monoclonal Ig in primary amyloidosis and myeloma amyloidosis

Serum and/ or urine	Plasma cells	Number of cases
None	$\gamma\lambda$	1
None	$\alpha\lambda$	1
None	λ	2
None	None	1
BJ λ	None	1
BJ λ	λ	2
BJ λ	$\gamma\lambda$	1
BJ κ	κ	1
IgG λ	$\gamma\lambda$	1
IgG λ	ND	1
IgG κ	$\gamma\kappa$	2
IgA λ	$\alpha\lambda$	1
IgM λ	$\mu\lambda$	1

$\kappa/\lambda = 3/12$.
ND, not determined.

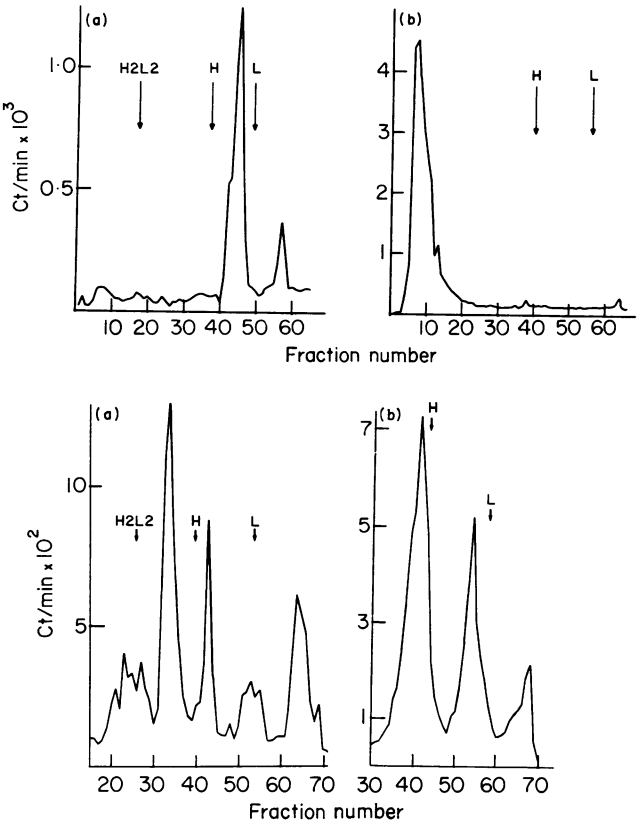

Fig. 2. Examples of unusual patterns: SDS-PAGE analysis of reduced and alkylated cytoplasmic extracts (a, 7·5% gel) and unreduced secretion (b, 5% gel) from the myeloma patient No. 10 (upper panel, except for small amounts of a fragment in the cytoplasm, the single detectable molecule was a 31 kD light chain secreted as a molecule of about 300 kD) and from the primary amyloidosis patient 15 (lower panel, assembly block of a monoclonal IgA λ: the major assembled molecule had an apparent mol. wt of 84 kD). This 84 kD molecule indeed contained H chains (since H chains were undetectable on unreduced gels) and it could not be a H2 dimer, which would have had a mol. wt of 120 kD. It contained L chains also, as shown by the comparison of the amounts of L chains on reduced and unreduced gels. Therefore, this 84 kD molecule is an HL half molecule. The pattern observed with secreted material was virtually superimposable except that the fragment was undetectable.

cell dyscrasias or have a urinary BJ whereas their plasma cells produce both H and L chains. In most patients with nonsecretory myeloma or BJ myeloma with intracellular H chain, the cells produce structurally abnormal Ig chains that are indeed secreted but not detectable in serum and urines (Preud'homme *et al.*, 1976; 1977a; 1980a). The hypothesis that such abnormal Ig might be responsible for tissue deposition in certain patients prompted us to perform biosynthesis experiments in LCDD and amyloidosis.

The present study combining IF and Ig biosynthesis experiments shows that free L chain secretion is a constant feature in primary and myeloma amyloidosis. We also found L chain fragments in nine of 10 cases. These results are strongly significant in view of the normal Ig biosynthesis pattern found in patients with non-myeloma secondary amyloidosis. Indeed, when dealing with a very small percentage of bone marrow plasma cells as in certain patients with primary amyloidosis, a major concern is that the finding of chains or fragments might be artefactual and result from proteolysis due to enzymes released by myeloid cells. Our data are in very good agreement with those recently published by Buxbaum (1986). The finding of fragments is special to amyloidosis since it is extremely unusual in common myeloma in our experience and others' (Zolla, Franklin & Scharff, 1970; Buxbaum, 1986). Whether fragments are synthetic products or result from the degradation of L chains, especially λ chains, synthesized as normal sized L chains is undecided. We rather favour the latter possibility since L chains from amyloidosis patients are especially susceptible to enzymatic degradation and since amyloid substance may be produced *in vitro* and probably *in vivo* by proteolysis of BJ proteins (Glenner *et al.*, 1971; Linke, Zucker-Franklin & Franklin, 1973; Epstein, Tan & Wood, 1974; Glenner, 1980; Durie *et al.*, 1982). In the present study, the largest amounts of fragments were found in those patients in whom there was no BJ in urines, conceivably because of post-secretory degradation and this is in accordance with this hypothesis.

The probable mechanism of amyloidosis therefore usually involves proteolysis of L chains secreted as intact molecules and this contrasts with the structural abnormalities found in the majority of patients with LCDD. However, certain patients affected with either of the diseases have similar features. As in LCDD, we found abnormally short H chains in two patients with primary amyloidosis, as reported in another case also (Buxbaum, 1986). In addition, in Patient 15, an assembly block of a monoclonal IgA at the level of HL half molecules was observed. Although the apparent mol. wt of these α chains was normal, this suggests a structural abnormality. On the other hand, AL type amyloid substance is usually made of L chain fragments containing mostly the variable region and a variable segment of constant region. However, amyloid deposits in some patients include entire or even enlarged L chains (Glenner, 1980). We herein report one case where the plasma cells secrete decamers of enlarged κ chains. The synthesis of large κ chains

and the secretion of tetrameric λ chains were also reported in one case each by Buxbaum *et al.* (1979) and Buxbaum (1986). These various abnormalities of Ig synthesis could have been observed in LCDD as well. We therefore believe that LCDD and AL type amyloidosis are closely related diseases featuring tissue deposition of monoclonal Ig derived material. The mechanisms appear to be different in 'typical' cases but they are almost indistinguishable in certain patients. This led us to suggest the possibility of the association of both processes in the same patients (Ganeval *et al.*, 1984), which was indeed observed in three recently reported cases (Jacquot *et al.*, 1985).

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