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The Major Internal Protein, p27, of a Retrovirus-like Particle Is Expressed in Blood Lymphocytes from Psoriatic Patients

By

O.-J. IVERSEN and A. B. DALEN

Department of Microbiology, Faculty of Medicine, University of Trondheim, Trondheim, Norway

With 6 Figures

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Summary

Retrovirus-like particles were isolated from the urine of a patient with psoriasis. The major internal protein, p27, in these particles was isolated by immunosorbent chromatography and gel filtration on a Sephacryl S-300 column in 6 M guanidine hydrochloride. The protein was purified to homogeneity as judged by SDS-PAGE. A hyperimmune serum with specificity for p27 was obtained by vaccination of a rabbit with purified p27 antigen. This antiserum was used to examine blood lymphocytes for the expression of p27 antigen by indirect immunofluorescence. Between 0.1 and 1 per cent of the lymphocytes obtained from patients with psoriasis showed a bright cytoplasmatic (and membrane) fluorescence while no p27 positive cells could be detected in the preparations from the healthy controls (frequency <0.01 per cent). Among the p27 positive psoriatic cells were lymphocytes with markers for T cells, B cells and NK cells.

Introduction

Subcellular particles with retrovirus-like morphology have been observed in psoriatic plaques, in the urine and blood from psoriatic patients, in the growth medium of cultivated cells from psoriatic lesions and in blisters from psoriatic lesions (2, 6). Immunological cross-reactivity has been demonstrated between the particles released from cultivated cells and those obtained from urine (13). The particles share certain characteristic features with retroviruses. They have a retrovirus-like morphology (2, 6), band at densities

between 1.15 and 1.18 g/cc in sucrose gradients (6, 13), and their protein composition resembles that of murine retroviruses, with an externally located glycoprotein, gp70, and three internal proteins p27, p15, and p12 (12, 13, 19). However, neither RNA-directed DNA polymerase (reverse transcriptase) activity nor polyadenylated high molecular weight RNA has been detected in these particles (14).

The particles have been observed in blood from psoriatic patients (6) which suggests production of particles in blood cells. In this work we describe a method for isolation of p27, the production of rabbit antibodies against p27 and we demonstrate expression of p27 antigens in blood lymphocytes from psoriatic patients.

Materials and Methods

Purification of p27 Antigen

Isolation of Virus-like Particles

Virus-like particles were obtained from the urine of a patient who had suffered from extensive psoriasis for 20 years (13). Two litre of urine was pelleted by centrifugation at $100{,}000 \times g$ for 90 minutes. The pelleted material was applied on the top of a sucrose gradient (20—65 per cent) and spun at $100{,}000 \times g$ for 18 hours. Virus-like particles, banding at densities between 1.15 and 1.18 g/cc (13), were collected, diluted in phosphate buffered saline (pH 7.2) (PBS) and pelleted by ultracentrifugation ($100{,}000 \times g$ for 90 minutes).

Purification of p27

The isolated urine particles were used for preparation of p27 antigen. The particles were disrupted in 0.1 per cent Nonidet P 40, and an aliquota of this material was labelled with ^{125}I for identification of p27 throughout the purification procedure. The labelling was performed according to the procedure of Bolton and Hunter (3). The isolation of p27 was essentially as described previously (13).

The disrupted urine particles were fractionated on a Con A Sepharose column (Pharmacia, Sweden). The material passing the column, the "Con A negative" material was applied on an immunosorbent column with rabbit antiserum against virus-like particles produced by cultivated psoriatic cells (6). Cross-reacting antigens were eluted in 1 M propionic acid, incubated in 8 M guanidine hydrochloride and fractionated on a Sephacryl S-300 column (Pharmacia) in 6 M guanidine hydrochloride (13). The fractions contaning p27 antigen was collected, dialysed against PBS and concentrated to 0.5 ml (Amicon concentration cell, YM 10 filter, Danvers, U.S.A.).

Electrophoretic Analysis

Analysis by SDS-PAGE in 10 per cent acrylamide gel (13, 26) was performed at the various steps in the purification procedure for identification of p27. The gels were sliced and counted in a gamma-counter (13). Standard proteins (SDS-PAGE Standard Low Molecular Weight, Bio-Rad) were labelled with ^{125}I and run in separate gels.

Rabbit Antiserum Against p27

Immunization Procedure

The purified p27 antigen obtained from the Sephacryl S-300 column was used for immunization of a rabbit. The antigen was emulsified with an equal volume

Freund's complete adjuvant (0.5 ml) and injected subcutaneously using multiple injection sites. The rabbit was boosted 6 weeks later with a corresponding amount of antigen (from 2 litre urine) in Freund's incomplete adjuvant. The animal was bled two weeks after the last injection.

Analysis of the Anti-p27 Serum

The antiserum was tested by indirect micro-ELISA. Microtitre plates [Organon Teknika (Greiner) Immulon Strips] were coated with purified p27 antigen in 0.1 M carbonate buffer (pH 9.6) overnight at 20° C. The amount of antigen applied corresponded to the amount obtained from 1 ml urine. The coated plates were washed in 3 per cent Tween 20 and incubated with anti-p27—or preimmune serum diluted in PBS at 37° C for 2 hours. Peroxidase conjugated swine anti-rabbit immunoglobulins (DAKO, Denmark) was used as second antibody. The wells were assayed with orthophenylenediamine for 30 minutes at 20° C and the reaction was stopped by addition of sulfuric acid (7). Absorption at 492 nm was recorded.

Fluorescence Microscopy

Clinical Specimens

Heparinized blood was obtained from patients with moderate to extensive psoriasis and from age matched healthy controls without known cases of psoriasis in their families.

Preparation of Blood Lymphocytes for Fluorescence Microscopy

Lymphocytes were isolated by the one step sodium metrizonate/Ficoll procedure (Lymphoprep, Nyegaard et Co., Oslo, Norway), and the cells were washed in PBS. The lymphocytes were adsorbed to coverslips that had been pretreated with 0.1 per cent polylysine (Sigma), and fixed in methanol or in 1 per cent formalin. The cells were incubated with rabbit anti-p27 serum diluted 1:60 in PBS at 20° C for 45 minutes, washed in PBS, followed by incubation with fluorescein conjugated swine anti-rabbit immunoglobulins (DAKO, Denmark) at 20° C for 20 minutes.

Identification of Lymphocyte Subpopulations

T cells, T_h cells, T_s cells and NK cells were identified by anti-leu-4, anti-leu-3, anti-leu-2 and anti-leu-11, respectively (Becton Dickinson's monoclonal antibodies). B cells were identified by BMA 0120 (Behringwerke AG, monoclonal antibodies).

Blood lymphocytes were isolated by the one step lymphoprep procedure (see above) and incubated with monoclonal antibodies in concentrations as recommended by the manufacturers at 20° C for 45 minutes. The cells were washed in PBS and incubated with Texas Red conjugated anti-mouse immunoglobulins (Amersham, U.K.) at 20° C for 20 minutes. The cells were then adsorbed to polylysine treated coverslips, fixed in methanol and labelled with anti-p27 antibodies and fluorescein conjugated anti-rabbit immunoglobulins as described above.

Results

Virus-like particles were isolated from the urine of a patient with psoriasis by sucrose gradient ultracentrifugation. The internal proteins of the particles were purified by ConA fractionation and immunosorbent chromatography (Fig. 1). The major internal protein, p27, was then

separated from the other proteins by gel chromatography on a Sephacryl S-300 column in 6 M guanidine hydrochloride, and purified to homogeneity as judged by SDS-PAGE (Fig. 2).

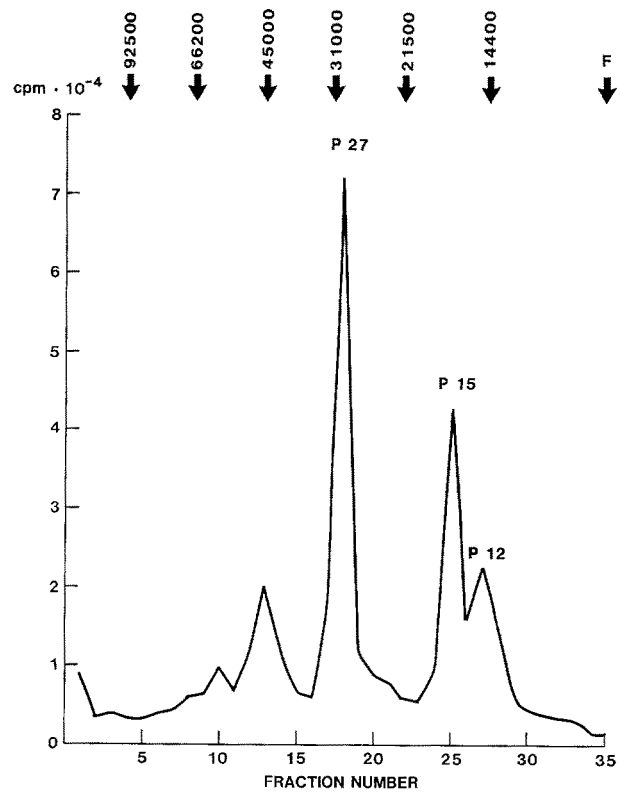

Fig. 1. SDS-PAGE of proteins labelled with ^{125}I of virus-like particles isolated from psoriatic urine by gradient ultracentrifugation. The proteins constitute the fraction which did not bind to Con A, but were adsorbed to an immunosorbent column with antibodies against virus-like particles obtained from cultivated cells from a psoriatic lesion (6)

Purified p27 antigen obtained from 4 litre urine was used for the production of rabbit anti-p27 serum. The serum was tested for anti-p27 activity by indirect micro-ELISA with purified p27 as antigen. The assay was performed with dilutions of the antiserum and of the preimmune serum. The antiserum showed anti-p27 activity in dilutions above 1:64,000 while no anti-p27 activity could be detected in the preimmune serum (Fig. 3).

The anti-p27 serum was used to examine blood cells for expression of p27 antigen. When blood lymphocytes from patients with psoriasis were fixed in methanol and incubated with anti-p27 serum followed by fluorescein conjugated swine anti-rabbit immunoglobulins, a bright cytoplasmatic

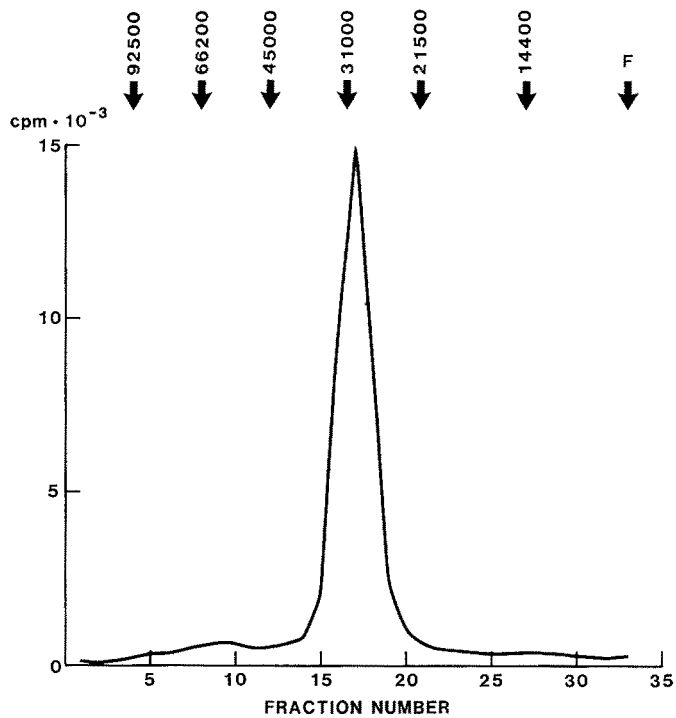

Fig. 2. SDS-PAGE of purified p 27 labelled with ^{125}I. The p 27 protein was isolated as described in the legend to Fig. 1 and was finally purified by gel chromatography on a Sephacryl S-300 column in 6 M guanidine hydrochloride

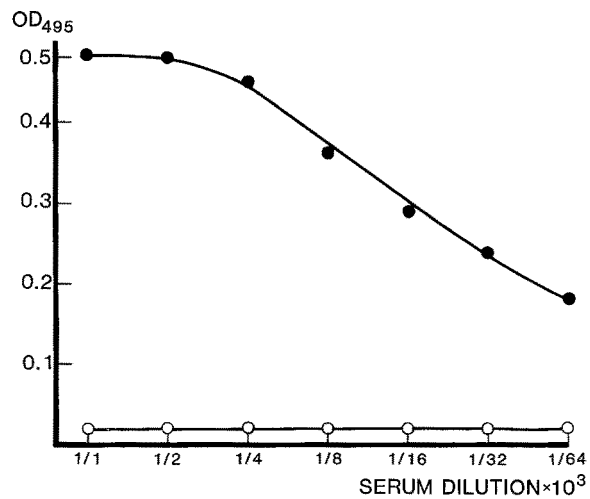

Fig. 3. Assay of the rabbit anti-p 27 serum (•) and preimmune serum (o) against purified p 27 antigen using the ELISA method (see Materials and Methods)

fluorescence appeared on approximately 0.5 per cent of the cells (Fig. 4). When the labelling was performed on cells fixed with formalin, the fluorescence was restricted to the outer membrane. The frequency of positive cells, however, was the same with the two methods. No fluorescence could be detected when the anti-p27 serum was substituted by the preimmune serum; thus we conclude that the reaction on the cells was due to anti-p27 antibodies.

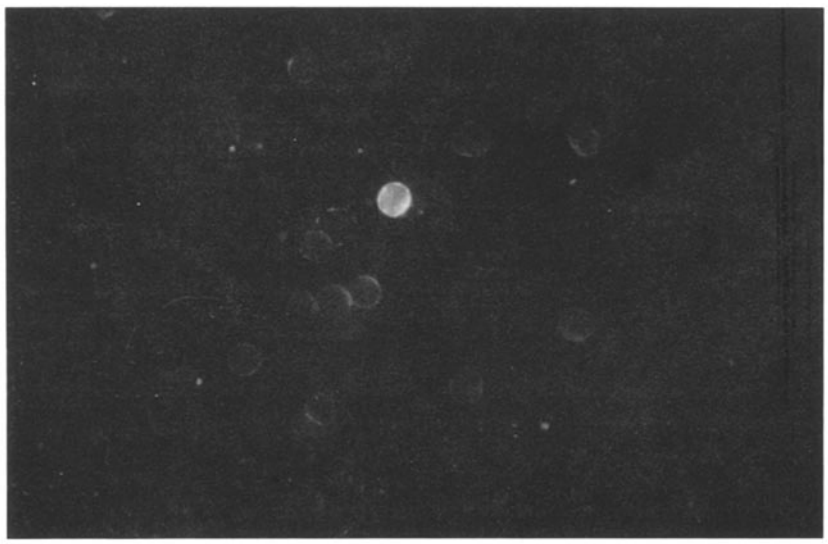

Fig. 4. Immunofluorescence micrograph of blood lymphocytes from a psoriatic patient. The cells were fixed in methanol and incubated with rabbit anti-p27 antibodies followed by fluorescein-conjugated anti-rabbit immunoglobulin antibodies

A double blind study was undertaken with 6 psoriatics with moderate to extensive disease and 6 healthy controls without known cases of psoriasis in their families. p27 positive lymphocytes were present in the 6 preparations obtained from the psoriatics, and the frequency of positive cells varied between 0.1 and 1 per cent. No positive cells were observed in the preparations from the controls (frequency below 0.01 per cent). Thus the expression of p27 on blood lymphocytes was restricted to the patient group.

To identify the lymphocytes producing p27 antigen a double labelling of the cells was performed. The lymphocytes were incubated with mouse monoclonal antibodies against markers for T cells, B cells or NK cells, followed by Texas Red conjugated goat anti-mouse immunoglobulins and rabbit anti-p27 serum followed by fluorescein conjugated swine anti-rabbit immunoglobulins. The fluorescence micrographs showed that the expression of p27 was not restricted to one population of lymphocytes (Fig. 5). The p27 antigen could be expressed in cells with markers for T, B or NK cells,

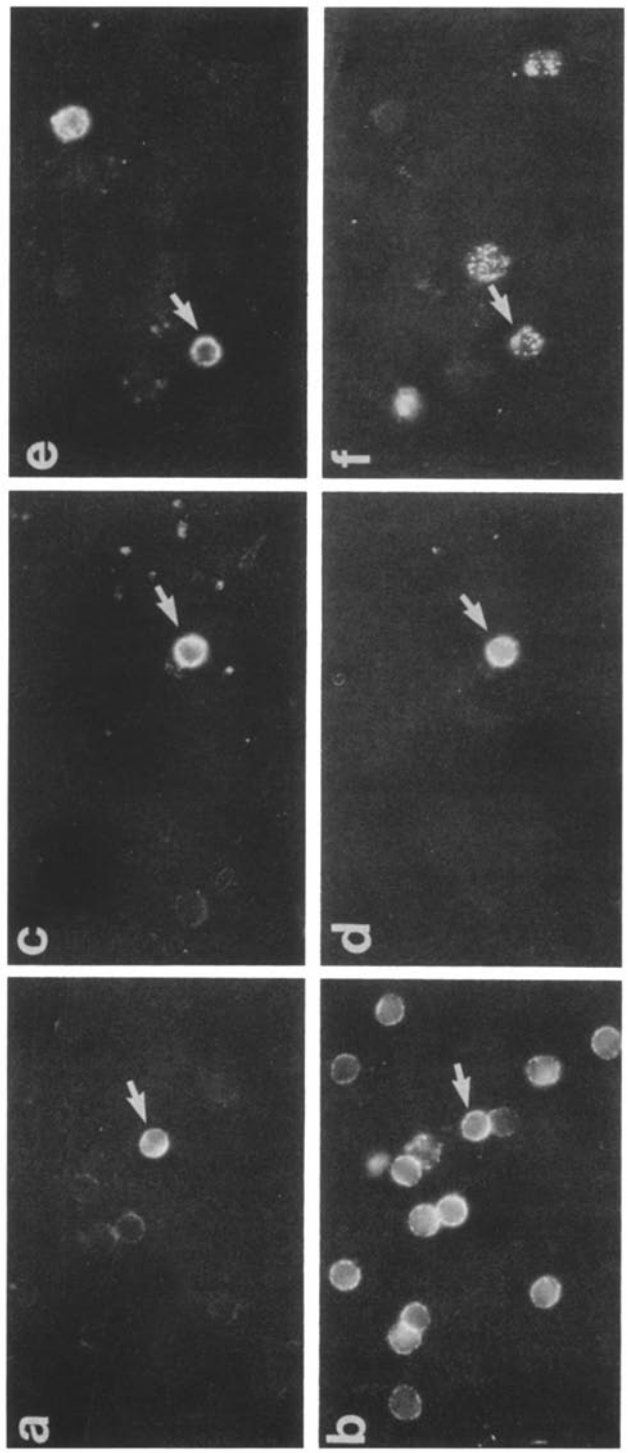

Fig. 5. Immunofluorescence micrographs of blood lymphocytes from a psoriatic patient doubly marked with rabbit anti-p 27 antibodies and mouse monoclonal antibodies against different markers for lymphocyte subpopulations. Binding of rabbit antibodies was visualized by fluorescein conjugates and mouse monoclonal antibodies by Texas Red conjugates. *a* shows a p 27 positive cell carrying T cell markers *(b)*, *c* shows a p 27 positive cell carrying B cell markers *(d)*, and *e* shows a p 27 positive cell carrying NK cell markers *(f)*

and the frequency of p27 positive cells was approximately the same for the three cell populations. Among the p27 positive T cells were cells with markers for T-helper/inducer cells as well as T cytotoxic/suppressor cells (Fig. 6).

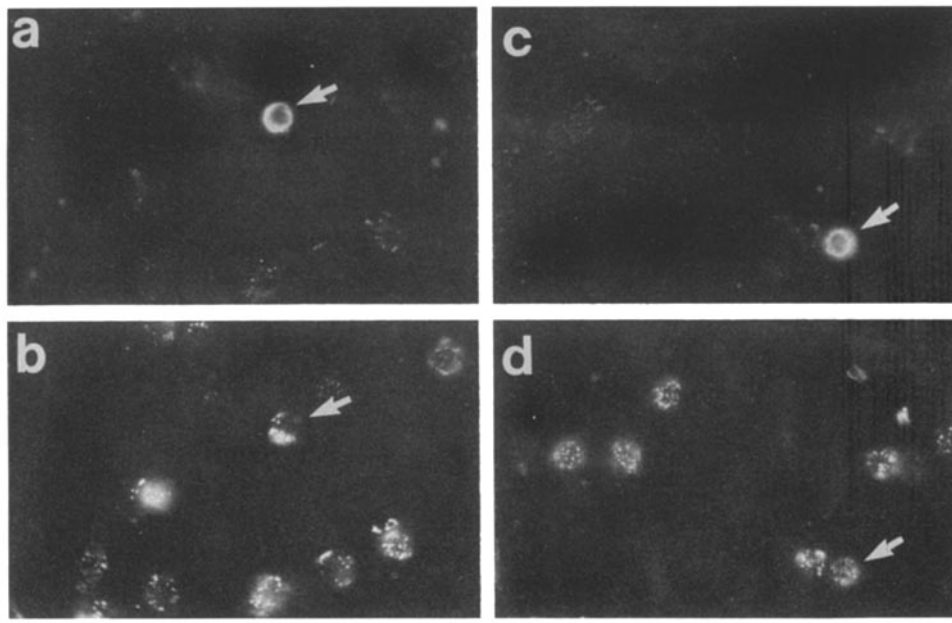

Fig. 6. Immunofluorescence micrographs of blood lymphocytes from a psoriatic patient double-marked with anti-p27 antibodies and monoclonal antibodies against T cell subpopulations (see legend to Fig. 5). *a* shows a p27 positive cell carrying T cytotoxic/suppressor cell markers *(b)*, and *c* shows a p27 positive cell carrying T helper/inducer cell markers *(d)*

Discussion

Subcellular particles resembling retroviruses have been isolated from patients with psoriasis (2, 6, 12, 13). The particles have a retrovirus-like morphology (2, 6), they have a buyoant density of 1.17 g/cc (6, 13) and have a protein composition characteristic for retroviruses with a surface glycoprotein, gp70, and three internal proteins, p27, p15 and p12 (12, 13).

A slow retrovirus infection could account for the main features of psoriasis. The disease is characterized by an enhanced proliferation of epidermal cells (27) which is a common feature of retrovirus infections, and a chronical inflammatory reaction with infiltration of mononuclear cells in the target organs is regularly seen in slow retrovirus diseases (20), and this is also one of the main characteristics of the psoriatic lesions.

In retrovirus infections the production of virus particles or virus antigens is not restricted to the clinical affected organs. Retroviremia and excretion

of virus particles in the urine are commonly seen. The subcellular particles associated with psoriasis have been demonstrated in serum immuncomplexes and in urine from patients with psoriasis (6, 13), indicating production of the particles in various cells.

The viremia which is seen in visna infections is always associated with production of virus in leucocytes (11). In this work we have demonstrated the expression of p27 antigen in blood lymphocytes from patients with psoriasis. The antigen was detected by indirect immunofluorescence with rabbit antiserum against purified p27 antigen and fluorescein conjugated swine anti-rabbit immunoglobulin antibodies. The antigen expression is not restricted to any particular subpopulation of the lymphoid cells, but the frequency of p27 positive cells is very low, 0.1—1 per cent which is in the same order of magnitude as that observed for expression of p30 in visna infections in sheep (20).

The anti-p27 serum did not react with blood lymphocytes obtained from healthy controls demonstrating that the antigen expression is a disease associated phenomenon.

The immune reactivity in psoriasis is altered. The cellular response to mitogens and antigens is depressed (5, 16) as well as the lymphokine production (17). The ratio of $T_{helper}/T_{suppressor}$ cells, however, are identical to that of normals (16). A slow retrovirus infection of lymphoid cells could account for the observed immunosuppression in patients with psoriasis (4, 22, 23, 24, 25).

Lymphocytes are the target cells of the retroviruses which have been related to human disease. HTLV 1 seems to play an important role in the aetiology of certain adult T cell leukemias (21), HTLV 2 is associated with hairy cell leukemia (15) and HTLV 3 is believed to play an important role in the development of AIDS (1, 8, 9, 10). T lymphocytes are the dominating target cells in the HTLV infections, but the infection of other lymphoid cells have been demonstrated (18). Whether HTLV does infect other organs is not known.

Based on protein analysis, the retrovirus-like particles isolated from patients with psoriasis show little relationship to HTLV I (13) and might represent a new type of human retroviruses.

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Authors' address: Dr. O.-J. IVERSEN, Department of Microbiology, Regional Hospital, N-7000 Trondheim, Norway.

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