Virchows Archiv A Pathol Anat (1989) 414:423–428

Virchows Archiv A
Pathological Anatomy and Histopathology

Immunohistochemical detection of proliferating cells in colorectal carcinomas and adenomas with the monoclonal antibody Ki-67. Preliminary data

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Summary. Twenty one cases of colorectal adenocarcinoma and six of adenoma have been studied using the monoclonal antibody Ki-67 which recognizes a nuclear antigen expressed by proliferating cells (PC). The quantitative evaluation of the stained nuclei showed that PC were more numerous in carcinomas than in adenomas although the difference did not reach a significant level. In each tumour, heterogeneity was noted. Furthermore, the superficial areas of both carcinomas and adenomas contained a greater number of PC than the deep. No difference was noticed in the various types and grades of differentiation for carcinomas. This preliminary report, compared with the only previous study of Shepherd et al. (1988) outlines the interest of the monoclonal antibody Ki-67 in the evaluation of growth fractions in colorectal tumours.

Key words: Colorectal carcinomas – Colorectal adenomas – Cell proliferation – Growth fraction – Monoclonal antibody Ki-67

Introduction

Several nuclear proteins that have recently been described are expressed in proliferating and transformed cells, but are absent in resting cells. Immunohistochemical studies of human tissues for the monoclonal antibody Ki-67 have proved useful in evaluating the proportions of proliferating cells in normal tissues and in benign and malignant lesions (Gerdes et al. 1983). Expression of this antigen occurs preferentially during late G_1, S, M and G_2 phases of the cell cycle, while cells in G_0 phase consistently lack the antigen (Gerdes et al. 1984a). This antibody reacts with a nuclear protein of proliferating cells in all human tissues. The highest incidence of stained nuclei was found in germinal centers of lymph nodes and spleen, cortical region of thymus, spermatogonies of testis, intestine crypt epithelium and basal cells of skin (Gerdes et al. 1983). Investigations in human malignancies have been limited, and include primarily leukaemias, non Hodgkin's lymphomas (Gerdes et al. 1984b; Gerdes et al. 1986; Pileri et al. 1986), breast carcinoma (Barnard et al. 1987), bone tumours (Vollmer et al. 1986) and neoplasms of the nervous system (Burger et al. 1986). Colorectal cancer is one of the few malignancies where a precursor lesion has been identified and most authorities now accept that an adenoma-carcinoma sequence takes place in the pathogenesis of the majority of large bowel adenocarcinomas (Muto et al. 1975; Hill et al. 1978). To our knowledge, only one study similar to ours has been reported by Shepherd et al. (1988) who studied 108 colorectal carcinomas with the monoclonal antibody Ki-67, using a semi-quantitative method. Only ten cases were assessed quantitatively by counting at least 2000 cells: they found a very good correlation between the quantitative and the semi-quantitative methods.

The purpose of the present study was to analyse colorectal adenocarcinomas and adenomas for the presence and distribution of Ki-67. Results of staining for Ki-67 were correlated with histological features for all the neoplasms, depth of invasion of the cancer, vascular spread and presence of metastases.

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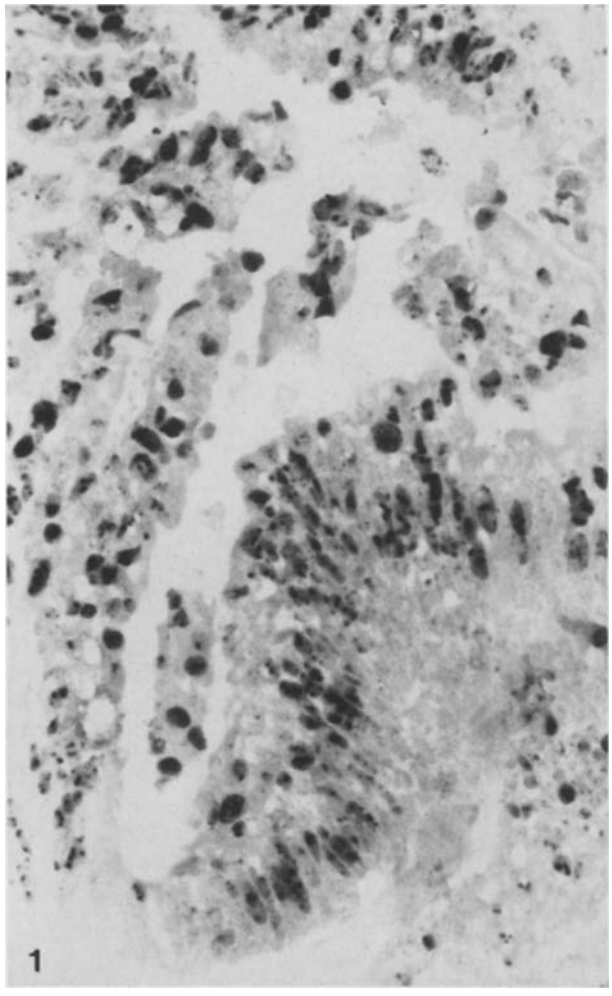
Material and methods

Twenty one cases of colorectal adenocarcinoma and six cases of solitary intestinal adenoma (from 3 cm to 17 cm diameter)



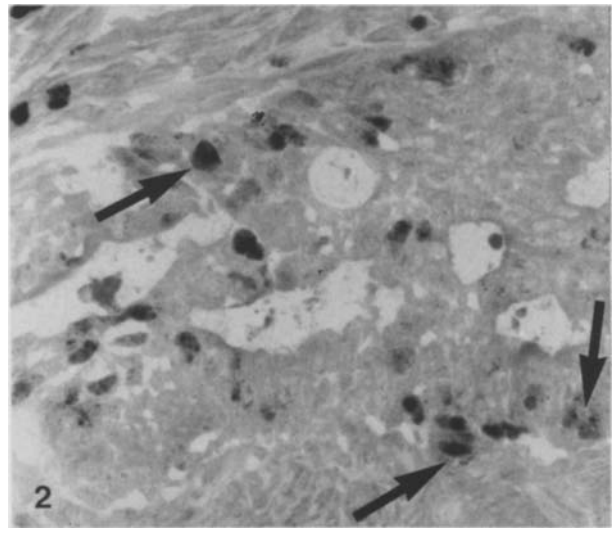


Fig. 1. Immunoperoxidase staining with Ki-67 antibody. Adenocarcinoma (group A) showing grade 3 proliferative index (positive nuclei >40%). 234×

Fig. 2. Immunoperoxidase staining with Ki-67 antibody. Carcinoma (group C) showing grade 1 proliferative index (positive nuclei <20%). 234×

were obtained from routine surgical procedures. In light microscopy three micron sections were made from paraffin embedded specimens and stained with haematoxylin-eosin-saffran (HES) and periodic acid Schiff (PAS). For immunohistochemistry fresh tumour samples were snap-frozen at −170° C in isopentane with liquid nitrogen. Six micron cryostat sections were cut and examined with the monoclonal antibody Ki-67 (Dakopatts, Glostrup, Denmark), diluted at 1:25. The three-step procedure (Ancelin et al. 1984), with 3-3′-diaminobenzidine tetrahydrochloride and appropriate negative controls were performed. After counterstaining with eosin and haematoxylin, each slide was evaluated for Ki-67 positivity in areas of neoplasms. Cells were considered as positive for Ki-67 only when definite brown staining of the nucleus was identified. Staining of stromal cells was easy to distinguish from nuclear staining of tumour cells as stromal cells only showed cytoplasmic staining.

A quantitative method for recording Ki-67 positivity was used by one observer, ignoring previous pathological findings. Sections were examined under a 40× objective. A camera lucida projected a grid on the field; an eyepiece graticule was sometimes used. Fields were randomly selected throughout each section, except in 9 cases (3 adenomas, 7 carcinomas) where superficial and deep parts could be easily characterized and accordingly differentiated. A mean of 9 fields and 1450 cells were counted in each tumour. The mean percentage of stained nuclei in the different fields of a tumour provided a Ki-67 index, or proliferation index (Pi). The mean Ki-67 index was compared in the different groups of tumours with a "t"-test or a variance analysis. A three stage grading (grade 1: Ki index <20%, grade 2: 20 to 40%, grade 3: >40%) was determined and compared with the other variables by a Chi II method.

The tumours were examined for morphological features according to the WHO Classification (Morson and Sobin 1976). Four groups of tumours were individualized: adenocarcinomas infiltrating the serosa with metastases (group A) and without metastases (group B), adenocarcinomas infiltrating the muscularis propria without serosal extension or metastases (group C) and adenomas (group D).

Results

All neoplasms studied exhibited positive nuclear staining for Ki-67 (Figs. 1, 2, 3, 4); the Pi for carcinomas values (Table 1) showed a wide variation between tumours, ranging from 7.92% to 48.44% (m: 28.59%, sd: 13.06%), which did not reflect invasive grade or tumour differentiation. No differences were observed between groups, or between moderately and poorly differentiated cancers. Tu-

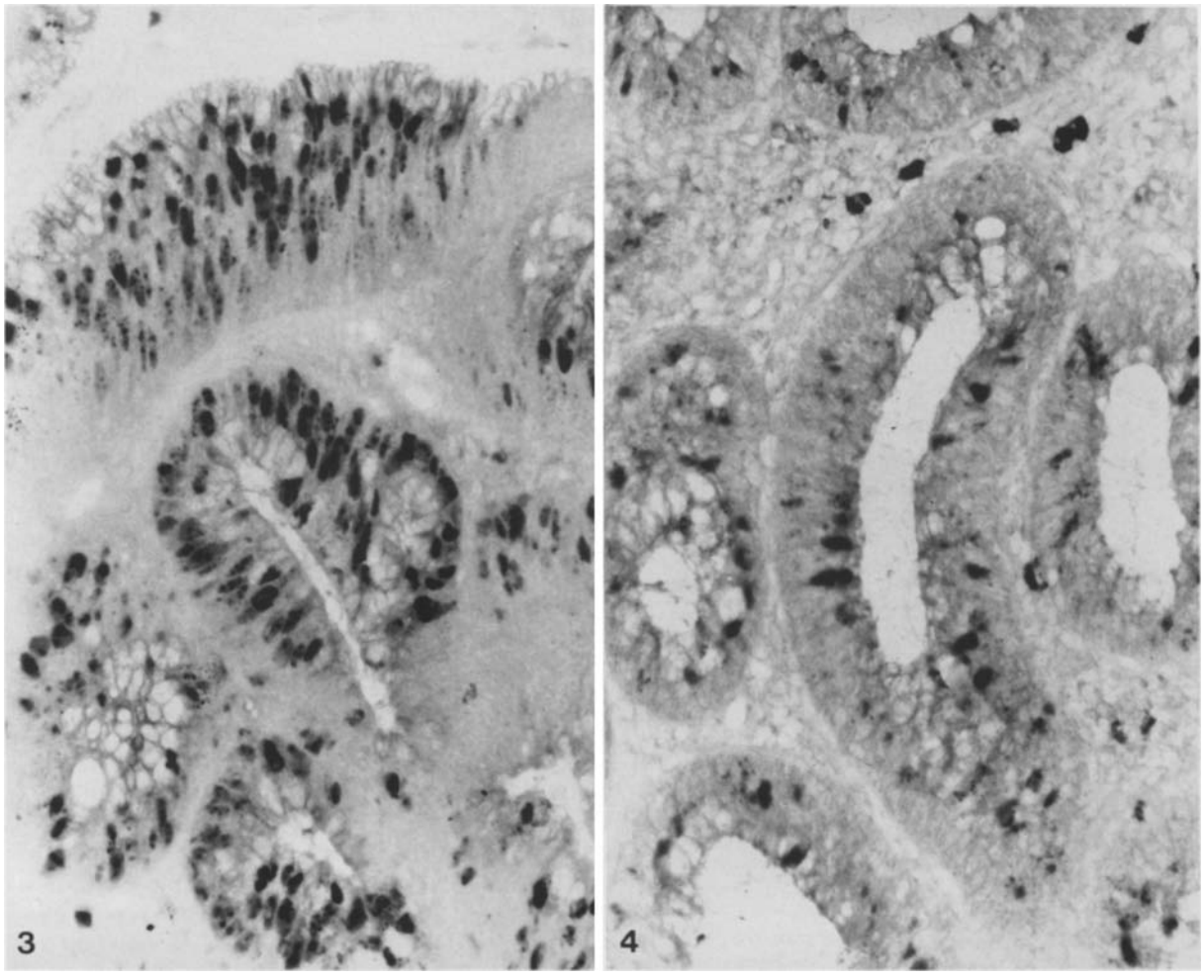


Fig. 3. Immunoperoxidase staining with Ki-67 antibody. Adenoma with severe dysplasia showing grade 2 proliferative index (positive nuclei between 20 and 40%). 234×

Fig. 4. Immunoperoxidase staining with Ki-67 antibody. Adenoma with moderate dysplasia showing grade 1 proliferative index (positive nuclei <20%). 234×

mours with and without vascular spread had a similar Pi (Table 2). The values of Pi for adenomas was less dispersed than among carcinomas (Table 1). There was neither a significant difference between moderate and severe dysplasia (Table 2) nor a significant correlation between Pi and tumour size. The comparison of adenomas and carcinomas emphasized a common point: there was a wide heterogeneity of the proportion of labelled nuclei in the different fields inside all tumours, reflected by the values for the standard deviation (Table 1). This variation seemed to occur at random in most cases where sections could not be oriented, as the fragments were taken from massive tumours, without normal intestinal structure. In the nine cases (six carcinomas-cases 2, 10, 12, 13, 14, 18− and three adenomas-cases 22, 23, 27−) where a differential count distinguished a superficial and a deep area, Pi was always higher near the surface (Table 3). For adenomas, Pi ranged from 30.8% to 40.1% (m: 35.93%, sd: 4.72%) in the superficial area, and from 5.52% to 12.10% (m: 7.82%, sd: 3.71%) in the deep field with a significant difference for $p=0.02$. In carcinomas, Pi ranged from 19.29% to 80.31% in surface and from 3.39% to 17.14% in depth, the difference was only significant in two single cases (10 and 12) in group B (Fig. 5). Though Pi seemed higher in carcinomas than in adenomas (Table 2), the difference did not reach significance owing to the small number of cases and the wide dispersion of values. It was also noteworthy to underline another interesting point: no adenoma and no group C carcinoma showed grade 3 labelling but Chi 2 test failed to demonstrate any signification in this difference.

Table 1. Comparison of Ki-67 immunoreactivity in groups A, B, C (carcinomas) and D (adenomas)

Case N°	Proliferation index		Case N°	Proliferation index	
	m	sd		m	sd
Group A			Group C		
1	46.57	13.63	17	11.85	7.29
2	22.48	9.03	18	18.79	20.64
3	32.66	17.53	19°	17.43	12.55
4	38.81	11.05	20°	35.65	14.48
5*	47.04	24.99	21°	33.72	12.73
6	7.92	5.04			
7	17.08	3.17			
8	27.18	9.67			
9	16.20	13.23			
Group B			Group D		
10*	24.19	16.57	22**	26.45	11.48
11	45.92	23.14	23**	18.55	19.52
12	45.10	30.02	24	20.56	11.21
13	17.17	8.78	25	20.32	14.42
14*°	18.34	9.21	26	13.16	7.19
15°	48.44	8.12	27	22.81	16.51
16°	40.51	7.43			

m, mean value measured in the different fields; sd, standard deviation; groups A and B, adenocarcinomas infiltrating the serosa respectively with and without metastases; group C, adenocarcinomas infiltrating the muscularis propria without serosal extension nor metastases

* adenocarcinomas with poor differenciation. All the others were moderately differentiated. ° adenocarcinomas without emboli. All the others had emboli; group D: adenomas;
** severe dysplasia. All the others showed moderate dysplasia

Table 2. Ki-67 derived proliferative grade of 22 carcinomas (moderately and poorly differentiated, without and with emboli) and six adenomas. Number of cases in each category

	Grade 1	Grade 2	Grade 3	Proliferation index	
				m	sd
Carcinomas					
Moderate differentiation	9	5	3	29.11	6.63
Poor differentiation	2	1	1	26.5	14.10
Without emboli	6	5	3	29.84	14.57
With emboli	3	1	3	28.52	12.82
Adenomas					
Moderate dysplasia	4	0	0	11.91	8.47
Severe dysplasia	0	2	0	28.77	10.13

m, mean value measured in the different fields; sd, standard deviation

Grades: grade 1: Ki index <20%; grade 2: 20–40%; grade 3: >40%

Table 3. Proliferation index in surface and depth in adenomas and carcinomas

	Adenomas (3 Cases)	Carcinomas (6 Cases)
Surface	m=35.93% (sd=3.72%)	m=37.25% (sd=22.41%)
Depth	m= 7.82% (sd=3.71%)	m=12.73% (sd= 6.80%)

m, mean proliferation index value; sd, standard deviation

Discussion

Rates of cellular proliferation and DNA synthesis have been shown to be important factors in the prognosis of many human malignancies (Bauer et al. 1987). Gerdes et al. (1983) have reported the production of a mouse monoclonal antibody designated Ki-67 that recognizes a nuclear antigen associated with cell proliferation. In breast carcinoma (Barnard et al. 1987; McGurrin et al. 1987) high numbers of Ki-67 positive cells were found in tumours with high mitotic rate, high nuclear rate and high histological grade. These correlations were similar to those previously reported for other measurements of cell cycle kinetics such as thymidine labelling index (McGurrin et al. 1987). A highly significant correlation has been demonstrated between the proportion of Ki-67 positive cells and the classification into high and low grade non Hodgkin's lymphomas according to the Kiel classification (Gerdes et al. 1984b; Weiss et al. 1987). Pileri et al. (1987) underlined that the immunocytochemical analysis of the proliferative rate with Ki-67 antibody in lymphoid tumours gave similar information to the radionuclide uptake assay, while Ki-67 activity represented a more sensitive test than the cytofluorimetric evaluation of the DNA content. In bone tumours (Vollmer et al. 1986), the results agreed with those of flow cytometric and autoradiographic studies on similar tumour entities. The monoclonal antibody Ki-67 was found to be handy and reliable tool for improved grading of bone tumours. Burger et al. (1986) used the monoclonal antibody Ki-67 in neoplasms of the nervous system: the number of stained nuclei in gliomas was in general agreement with the histological grade and known biological behaviour of the lesions. Only one previous study, by Shepherd et al. (1988) can be compared with ours. In the present work the number of cases was smaller (22 carcinomas) but each of them was studied with a quantitative method. On the whole, our results were in accordance with theirs: there was no difference in Ki-67 labelling according to carcinoma differentiation, parietal spread and presence or absence of vascular spread and metastases. We also

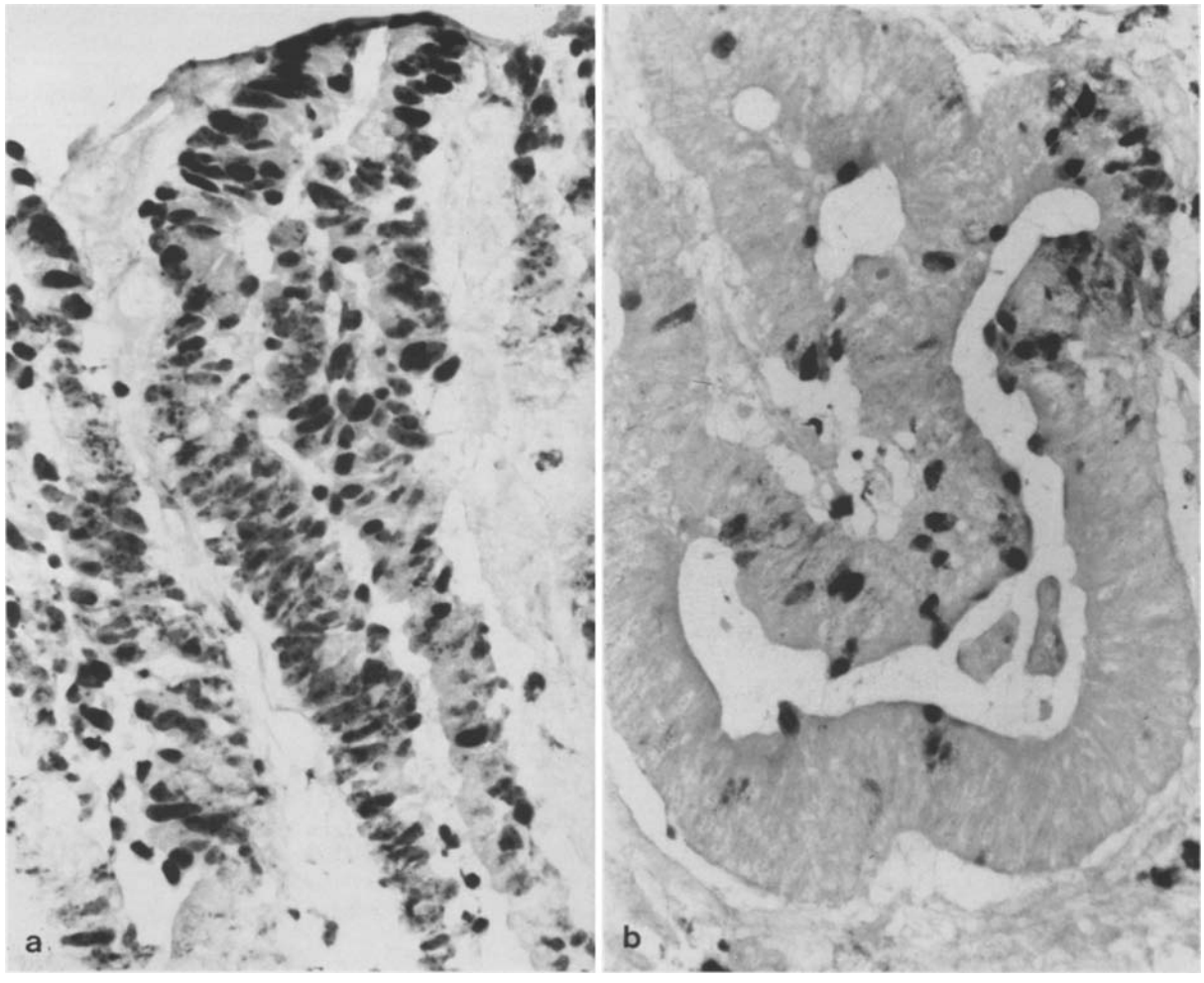


Fig. 5. Immunoperoxidase staining with Ki-67 antibody. Adenocarcinoma (group B). Grade 3 proliferative index (positive nuclei >40%) in the superficial area (**a**) and grade 1 proliferative index (positive nuclei <20%) in the deep part (**b**). 234×

observed a great heterogeneity of labelling within individual tumours but the dispersion of the proliferative activity through the different cases was more marked in their series, ranging from 1 to 80% (semi-quantitative score) than in ours which showed Ki-67 index from 8 to 48% (quantitative score) and allowed us to distinguish three grades among which tumors were almost evenly distributed. Our grades 1, 2 and 3 corresponded respectively to Shepherd et al. (1988)'s grades 1+2, grade 3 and grade 4. We found no equivalent to their grade 5. However, a precise comparison was not possible because the classification of the carcinomas was quite different in the two series; Shepherd et al. (1988) had a higher proportion of high grade tumours.

The original finding in the present work lies in the study of the six adenomas. On the whole the labelling index was less varied and lower than in carcinomas, ranging from 13% to 26%, which corresponded to grade 1 and 2, no case being in grade 3. However inside each tumour, proliferative activity was heterogeneous, as in carcinomas. An apparently striking difference was observed between moderate dysplasia (grade 1) and severe dysplasia (grade 2) but with the small number of cases, these data were not found to be statistically significant. In the nine cases (three adenomas, six carcinomas) where this variable could be measured, Ki-67 index was higher in superficial areas than in deeper parts of the tumours: this variation was significant for the three adenomas but only for two of the carcinomas.

From these data, Ki-67 nuclear labelling seems to be an interesting method for the study of colorectal carcinomas and adenomas. Prospective studies of a greater number of cases with follow-up of patients, in order to establish the degree of correlation between survival and Ki-67 nuclear labelling in carcinomas is necessary.

Acknowledgements. This study was supported by the "Ligue Nationale contre le Cancer" (Comité de Paris), the "Conseil Scientifique de la Faculté de Médecine Lariboisière-Saint-Louis" (Université Paris VII) and the "Association pour la Recherche sur le Cancer" (ARC, Villejuif, France). We thank Elisabeth Skrobala and Marie-France Bouvet for their technical assistance, and Isabelle Coquart for secretarial support.

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Received May 16, 1988/Accepted January 17, 1989